Cytotoxic Metal Complexes With N-Heterocyclic Carbenes Containing 1,2,4-Oxadiazole Substituents

Von der Fakultät für Lebenswissenschaften

der Technischen Universität Carolo-Wilhelmina zu Braunschweig

zur Erlangung des Grades eines

Doktors der Naturwissenschaften

(Dr. rer. nat.)

genehmigte

D i s s e r t a t i o n

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aus Pascani, Rumänien

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eingereicht am: 16.08.2017

mündliche Prüfung (Disputation) am: 01.12.2017

Druckjahr 2018

Vorveröffentlichungen der Dissertation

Teilergebnisse aus dieser Arbeit wurden mit Genehmigung der Fakultät für Lebenswissenschaften, vertreten durch den Mentor der Arbeit, in folgenden Beiträgen vorab veröffentlicht:

Publikationen

C.-V. Maftei, E. Fodor, P. G. Jones, C. G. Daniliuc, M. H. Franz, G. Kelter, H.-H. Fiebig, M. Tamm, I. Neda, Novel 1,2,4-oxadiazoles and trifluoromethylpyridines related to natural products: synthesis, structural analysis and investigation of their antitumor activity, *Tetrahedron* **2016**, *72*, 1185-1199.

C.-V. Maftei, E. Fodor, P. G. Jones, M. Freytag, H. M. Franz, G. Kelter, H.-H. Fiebig, M. Tamm, I. Neda, *N*-heterocyclic carbenes (NHC) with 1,2,4-oxadiazole-substituents related to natural products: Synthesis, structure and potential antitumor activity of some corresponding gold(I) and silver(I) complexes, *Eur. J. Med. Chem.* **2015**, *101*, 431-441.

C.-V. Maftei, E. Fodor, P. G. Jones, C. G. Daniliuc, M. H. Franz, G. Kelter, H. H. Fiebig, M. Tamm and I. Neda, Novel bioactive 1,2,4-oxadiazole natural product analogs. Synthesis, structural analysis and potential antitumor activity, *Rev. Roum. Chim.* **2015**, *60*(1), 75-83.

C.-V. Maftei, E. Fodor, P. G. Jones, M. H. Franz, G. Kelter, H. Fiebig and I. Neda, Synthesis and characterization of novel bioactive 1,2,4-oxadiazole natural product analogs bearing the *N*-phenylmaleimide and *N*-phenylsuccinimide moieties, *Beilstein J. Org. Chem.* **2013,** *9*, 2202-2215.

C.-V. Maftei, E. Fodor, I. Mangalgaiu, P. G. Jones, C. G. Daniliuc, M. H. Franz, I. Neda, Synthesis of 3-*tert*-butyl-5-(4 vinylphenyl)-1,2,4-oxadiazole using two different pathways, *Rev. Roum. Chim.* **2010,** *55*, 989-994.

Tagungsbeiträge

C.-V. Maftei, E. Maftei, M. H. Franz, I. Neda: Cinchona Alkaloid Based Key Structures: Quincorine And Quincoridine And Derivatives Thereof (Vortrag). The 34th National Chemistry Conference October 4-7, 2016, Călimăneşti-Căciulata, Vâlcea, Romania.

Posterbeiträge

C.-V. Maftei, E. Maftei, M. H. Franz, M. Tamm and I. Neda: *N-Heterocyclic Carbenes related to Natural Products: Synthesis, Structure and Potential Antitumor Activity of some Corresponding Gold(I) and other Noble Metal Complexes (Poster)*. Modern Biotechnologies

in Sustainable Development of the Danube Delta, May 31-June 2, 2016, Murighiol, Tulcea, Romania.

C.-V. Maftei, E. Fodor, M. H. Franz, I. Neda: *Novel Silver and Gold N-Heterocyclic Carbene Initiators in the Ring-Opening Polymerization of L-Lactide (Poster)*. 15th International Conference on Polymers and Organic Chemistry, June 10-13, 2014, Timisoara, Romania.

Acknowledgements

The path taken for accomplishing this PhD has been a really life-changing experience and it would not have been accomplished without the help and guidance given by many people.

First and foremost, I would like to thank my scientific advisors Prof. Matthias Tamm and Prof. Ion Neda, for giving me the opportunity to develop my PhD thesis at the Technical University Braunschweig. I deeply appreciate them for the time, advices and funding contributions made in order to make my PhD experience in the same time valuable and exciting. Without their counselling and permanent feedback this PhD would not have been accomplishable.

I would like also to express my gratitude towards Dr. Martin Heiko Franz for his scientific advices and suggestions during research discussions and for being always encouraging with my work. He always had invaluable answers to my difficult questions and unresolved problems.

Furthermore I would like to thank my reading committee members: Prof. Matthias Tamm and Prof. Henning Hopf for their time, concern and useful comments. Also, I would like to express my appreciation to the third member of my oral defense committee, Prof. Marc D. Walter, for his time and insightful questions.

I also want to show my gratitude to the whole analytical department. Special thanks to Ingo Kurz for being patient and helpful with me during the ESI measurements. In this context, I would like to thank also the crystallographic help from Prof. Peter G. Jones and Dr. Constantin Daniliuc.

The financial support from InnoChemTech GnbH Braunschweig during this PhD work is highly appreciated.

In the end, I would like to thank my family for their unconditional love and support in all my pursuits, especially to my parents who raised me to appreciate the spirit of nature and science.

Finally but not least I would like to thank my loving, encouraging, understanding and patient wife Elena, who has been by my side during this long journey, and without whom, everything would have been more difficult.

I. Introduction

I.1. General Motivation

I.1.1. Heterocycle molecules with biological activity

Heteroatomic ring systems are one of the essential parts of most biologically active drug molecules. The main properties of the heteroaromatic rings are correlated with the fact that they can furnish analogies to the biologically active molecules present in the human body. The hormones, nucleic acids, neurotransmitters, all have in their structures at least one heteroaromatic ring.

Oxadiazoles (furodiazoles) are five-membered ring derivatives which include one oxygen and two nitrogen atoms in their cyclic system. Depending on the position occupied by the oxygen and nitrogen atoms in the ring, oxadiazoles are categorized in four different groups[1]: 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole and 1,3,4-oxadiazoles (Figure 1).

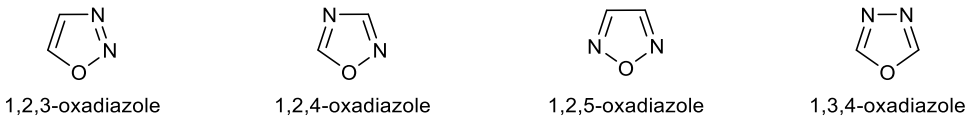


Figure 1. Oxadiazole systems.

The **1,2,3-Oxadiazole** ring system is stabilized mainly in the diazo-oxide form. One of the important features of these compounds is their ability to stabilize themselves in a higher oxidation state. Sydnones and sydnonimines derivatives are the best representantives of this class.[2] Taking into consideration the biological activity of these compounds, two of the sydnonimines derivatives, Molsidomine and Sydnocarb, must be acknowledged (Figure 2). Molsidomine has a long-lasting effect as vasodilatation agent and decreases the effort of the heart in cases of ischemic heart disease. It behaves similar to nitroglycerine in the treatment of angina pectoris.[3] Sydnocarb acts on the central nervous system and has been used as a psycho-stimulant.[4] Other sydnones and sydnonimines are reported to have anti-inflammatory, antitumor, antibacterial, analgesic and antipyretic activity.[5]

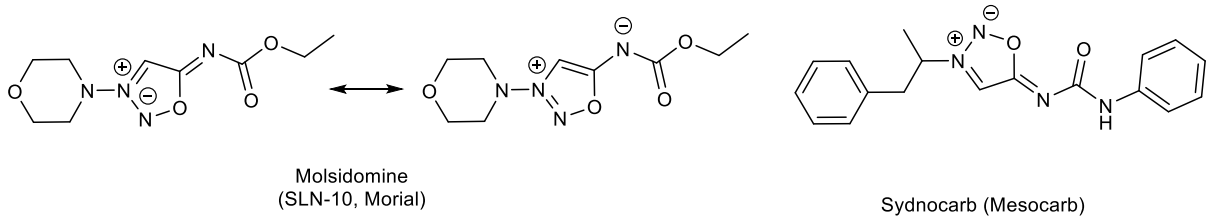


Figure 2. Bioactive 1,2,3-oxadiazoles.

1,2,5-Oxadiazoles (Furazans) and their corresponding oxides (furoxans and benzofuroxans) are well known for their biological properties. This class of compounds is reported to have antimicrobial, immunosuppressive, vasodilator or anticancer activity.[6] In recent studies on the development of new anti-ulcer drugs, calcium channel modulators or vasodilator compounds, the furoxan and the benzofuroxan systems were designed along with other traditional drug moieties in a single molecule.[7] The most conclusive examples are the furoxan and the benzofuroxan derivatives bearing as substituent dihydropyridine in an attempt to develop calcium channel modulators (Figure 3). Isradipine, a calcium channel blocker of the dihydropyridine class has selective effects on coronary arteries and the sinus node,[8] in order to reduce the risk of stroke and heart attack. The nitro derivative has an opposite effect, hence, it is a calcium channel activator.[9]

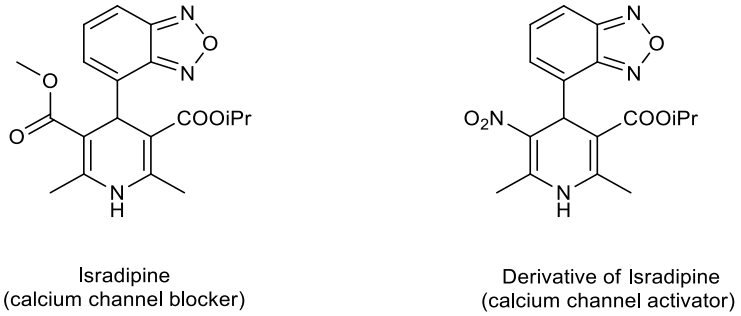


Figure 3. Bioactive 1,2,5-oxadiazoles.

Many furoxan derivatives were tested for antibacterial, antiprotozoal (*T. vaginalis* and *Entamoeba histolytica*), antifungal and mutagenic activities.[10]

1,3,4-Oxadiazoles are considered good biomimetic and reactive pharmacophores.[11] Because of their biological activities,[12-14] these heterocycles are used also in the development of pesticides,[15] compounds with anti-peripheral vasomotility,[16] central nervous system stimulants, anti-inflammatory and hypotensive drugs,[17] insecticidals,[18] bactericidals,[19] analgesics, anticonvulsives, antiemetics, diuretics,[20] hypoglycemics,[21] muscle relaxants,[22] herbicidals[23] and fungicidals.[24]

1,2,4-Oxadiazoles [furo(ab$_1$)diazoles], were reported for the first time by Tiemann and Krüger[25] in 1884. For a long time, this heterocycle was just mentioned in a small number of publications. However, since 1960, the 1,2,4-oxadiazoles have received more attention from the chemistry community due to their unusual tendency to go through molecular

rearrangements. The application of this heterocycle in medicinal and material chemistry has led to an increasing number of publications dealing with 1,2,4-oxadiazoles.

I.1.2. Medicinal aspects of organic and organometallic compounds

I.1.2.1. 1,2,4-Oxadiazole abundance in nature

To the best of our knowledge, there are only a few examples of natural products possessing a 1,2,4-oxadiazole core or structure based on it. The 3-substituted indole alkaloids, Phidianidines A and B (Fig. 4), were isolated by Carbone *et al*. from the aeolid opisthobranch *Phidiana militaris*.[26] They are selective inhibitors of dopamine transporter DAT and partial agonists of the μ opioid receptor.[27] Moreover, these selective molecules are attractive as CNS targets because neither Phidianidine A nor B are cytotoxic. A further example is quisqualic acid (Fig. 4). This metabolite was obtained from the seeds of *Quisqualis indica* and *Q. fructus*[28] and is a strong agonist for AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors and group I metabotropic glutamate receptors.[29]

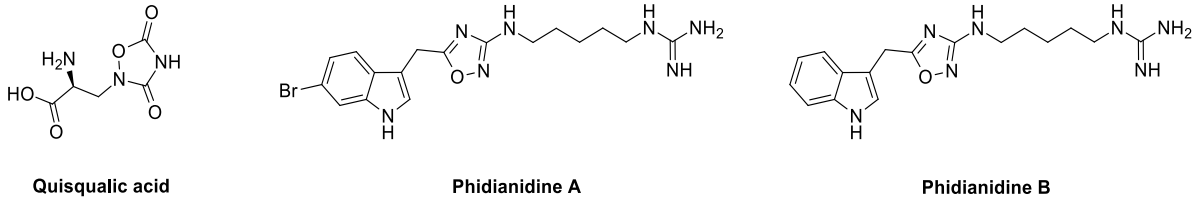


Figure 4. Natural products possessing 1,2,4-oxadiazole core.

I.1.2.2. Medicinal chemistry of 1,2,4-oxadiazoles

The five-membered heterocyclic 1,2,4-oxdiazole motif is of general and increasing synthetic and pharmacological interest and forms an important constituent of biologically active compounds including natural products.[30] Sawyer et al. have described such compounds as bioisosters for amides and esters.[31] The hydrolytic and metabolic stability of 1,2,4-oxadiazoles is increased as a result of the five-membered ring. Since the neutral 1,2,4-oxadiazole system can act as a bioisoster for amide and carboxylic acid groups, researchers implemented this structural motif in bioactive molecules. Several bioactive peptides (hormones analogues, enzyme inhibitors or neurotransmitters) undergo fast hydrolysis by peptidase enzymes present in the human body which limits their therapeutic properties. To surmount this obstacles efforts have been undertaken to replace the amide moiety by the 1,2,4-oxadiazole system.

A series of 1,2,4-oxadiazole-5-one and 1,2,4-oxadiazole-5-thione, isosters of nicotinamide and pyrazinamide, were reported to have antitubercular activity (Figure 5).[32]

Pivaloyloxymethyl derivatives of these isosters were synthesized with the purpose of increasing the lipophilicity and thereby improving the cellular permeability. These compounds are supposed to be modified by esterases to the active species after penetration of the mycobacterial cell wall. The potency of these compounds was found to be up to 16 times higher than pyrazinamide.

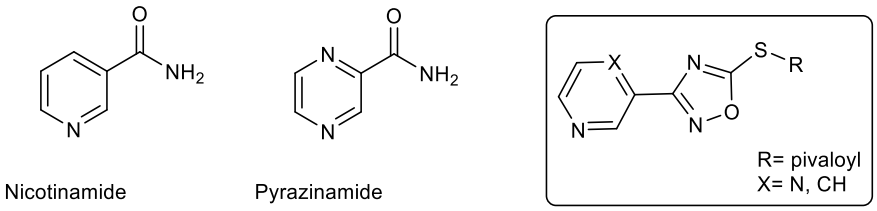


Figure 5. 1,2,4-Oxadiazole isosters for nicotinamide and pyrazinamide.

Another bioactive 1,2,4-oxadiazole derivative was isolated by Hennen *et al.* (Fig. 6).[33] They have compared the specific absorption rate (SAR) of Ribavarin, Tiazofurin and their analogues as antiviral and antineoplastic agents. The results showed that the 1,2,4-oxadiazole-containing compound induces 46% inhibition of leukemia L1210 and 43% inhibition of Leukemia P388 at $1x10^{-4}$ M in cell culture.

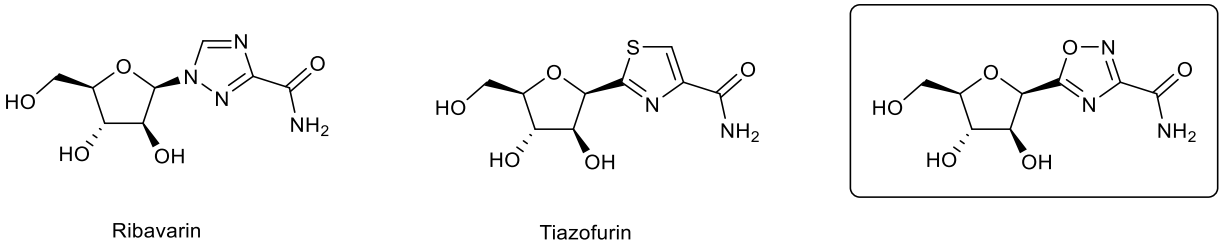


Figure 6. An 1,2,4-oxadiazole isoster for Ribavarin and Thiazofurin.

Saunders et al.[34] established the importance of the 1,2,4-oxadiazole system as a bioisoster for ester derivatives of muscarinic derivatives. The oxadiazole-substituted muscarinic ligands (Fig. 7) were found to be efficient against Alzheimer's disease.

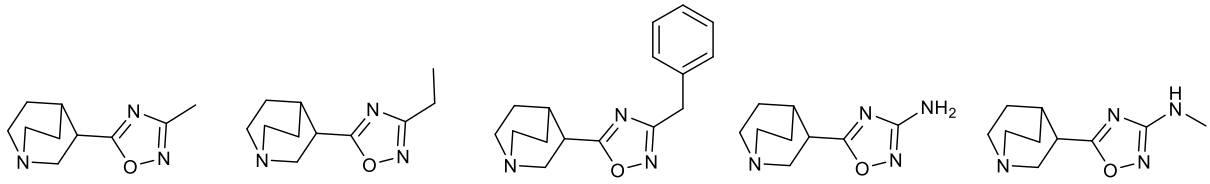


Figure 7. 1,2,4-Oxadiazole bioisosters for esters derivatives of muscarinic derivatives.

Oxadiazole [(-)-2*β*-(1,2,4-oxadiazol-5-methyl)-3*β*-phenyltropane] (Fig. 8) is a phenyl tropane derivative acting as a stimulant drug with almost five times higher activity than cocaine. In addition it inhibits the monoamine reuptake.[35]

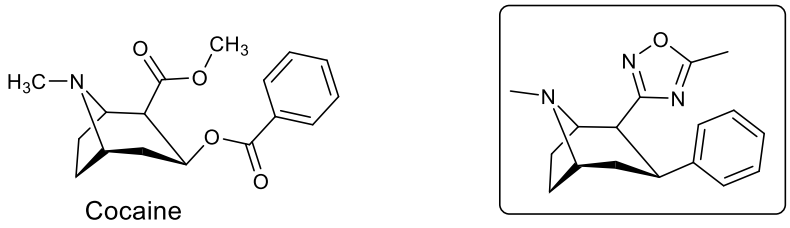


Figure 8. 1,2,4-Oxadiazole-phenyltropane derivative with stimulant effect.

The oxadiazole ring system is also present in the molecular structure of some commercial drugs with different therapeutic properties, such as antiinflammatory, vasodilator, anesthetic or antitussive (Figure 9).[36]

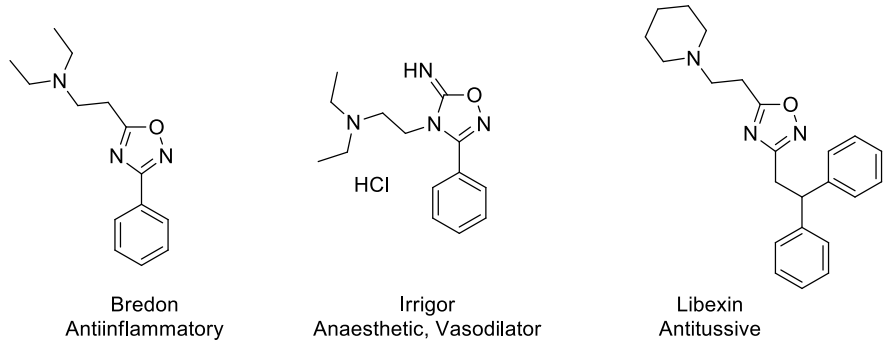
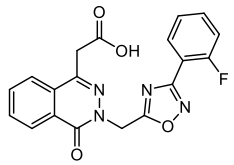
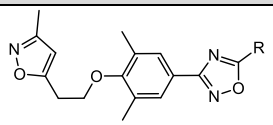
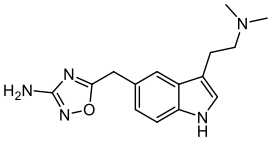
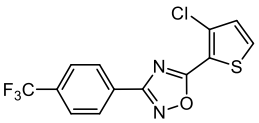
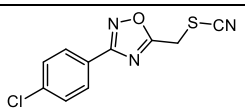
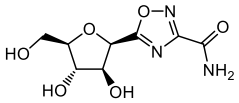
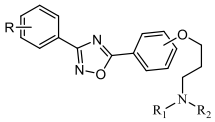
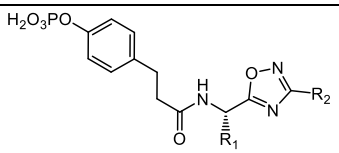
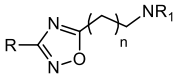
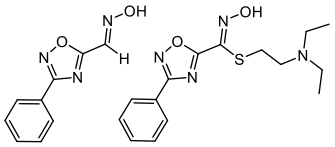
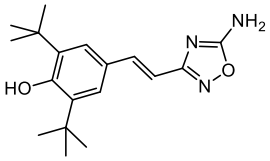
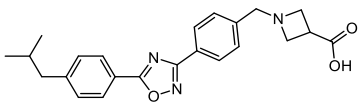
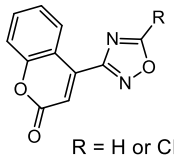
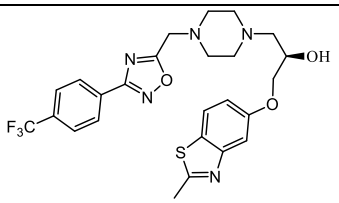
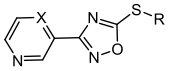
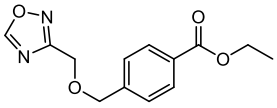


Figure 9. Examples of drugs containing the 1,2,4-oxadiazole unit.

Other biological properties of 1,2,4-oxadiazole containing molecules, with a wide spectrum of action, from anti-parasites to anti-inflammatory activity, are summarized in Table 1.

Table 1. Literature reported bioactive 1,2,4-oxadiazole.

1,2,4-Oxadiazole derivative	Biological properties	1,2,4-Oxadiazole derivative	Biological properties
	-strong aldose reductase inhibitor -analog for the marketed drug Zopolrestat [37]	 R= alkyl, aryl, alkylamine, OH, NH_2	-bioisosters of esters reported to possess anti-rhinoviral activity [45]
	-potent 5-HT1D agonist analog for Sumatriptan, drug used in the treatment of migraine [38]		-apoptosis inducer. -antitumoral activity against breast and colorectal cancer cell lines [46]
	-activ against kinetoplastid parasites (Leishmania donovani, Trypanosoma brucei) [39]		-antiviral and antineoplastic agent [33]
	-Interleukin-8 (IL-8) antagonists that could lead to powerful antiinflammatory agents [40]		-strong SH2 inhibitors of the tyrosine kinase ZAP-70 [31]
 R= aryl or arylalkyl n= 0,1,2,3 NR_1= Substituted amines	-antispasmodic and antitussive activity [41] -analgesic and anti-inflammatory activity [42]		-strong inhibitor agent of human Ache [47]
	-active against in the rat carrageen footpad edema and mycobacterium footpad edema [43]		-excellent Sphingosine-1-phosphate-1(S1P1) receptor agonist with very good selectivity against S1P2 and S1P3 receptor subtypes (S1P1 IC50 = 0.6 µM) [48]
 R = H or CF_3	-inhibitors for Trypsin Glucuronidase and Soybean Lipoxygenase -good antioxidant character -antiinflammatory activity in the rat carragenan paw edema assay [44]		-good palm CoA inhibitor (IC50=0.38 µM) [49]
 R= pivaloyl X= N, CH	-isosteres of nicotinamide and pyrazinamide, reported to have antitubercular activity [32]		-hypocholestermic agent [50]

Furthermore, 1,2,4-oxadiazoles are widely employed in synthetic chemistry e.g. in the search for antitumor agents. Cancer consists of more than one hundred different diseases, all characterized by uncontrolled growth and spread of abnormal cells. In this context, the identification of drugs inducing apoptosis represents an attractive approach for the discovery of new anti-cancer agents. By means of a high-throughput screening (HTS) assay, 1,2,4-oxadiazole **A** (Fig. 10) was found to act as an apoptosis agent.[46] A series of 1,2,4-oxadiazole-5-carboxamides **B** have been synthesized and tested as inhibitors of the glycogen synthase kinase 3 (GSK-3), a key regulator of both differentiation and cellular proliferation.[51]

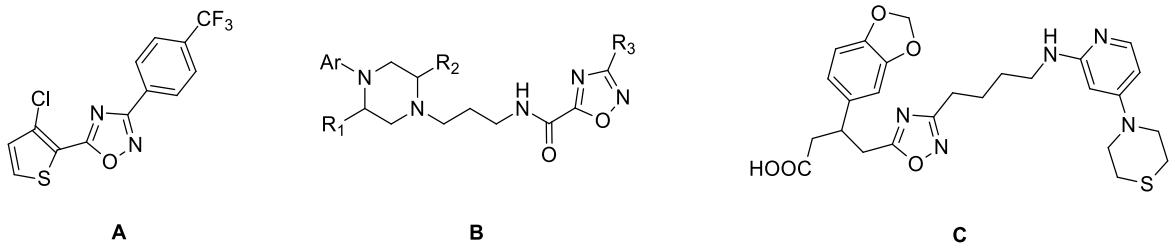


Figure 10. Examples of antitumoral drugs containing 1,2,4-oxadiazole core.

An alternative anti-tumor strategy involves the inhibition of the tumor growth, such as angiogenesis. Integrin $\alpha_v\beta_3$ is a receptor that has been found on the surface of many tumor cells and it recognizes the arginine–glycine–aspartic acid (RGD) sequence. Antagonists of this receptor are able to inhibit angiogenesis. 1,2,4-Oxadiazole-butanoic acids such as **C** were tested as nonpeptidic analogues of $\alpha_v\beta_3$ antagonists.[52]

I.1.2.3. Cancer pathology

A tumor is a cell modification generated by an alteration in the metabolism or by a genetic mutation. The abnormal growth of cells (when the cell cycle and cellular role is not working properly) and their proliferation in a chaotic fashion are defined as cancer.

The etymology of *cancer* has the origins from Greek word *carcinos*. The Greek physician Hippocrates used this term to report characteristics of the pathology.

Although cancer is noted from ancient times and many research programs were developed in the last decades in the attempt to find a cure, it is still responsible - according to World Health Organization - for more than 12 million deaths per year, making it the second most frequent cause of death after heart diseases.[53,54]

Human living conditions and the aging of the human body are considered the two most important factors responsible for cancer development. The malignant tumours related to a hereditary origin is just around 5%.[55] This illness is strongly correlated with the living environment of individuals. The most frequent causes for the development of different forms of tumours involves live style (drugs, cigars, alcohol), working conditions (heavy metal, asbestos, benzene) or a non-balanced alimentation.[56] Overall the human body can be affected by hundreds of cancer types of which lung cancer is among the most frequently observed. At the opposite end, kidney or pancreatic cancers belong to the less common ones.

Based on the tissue affected or the initial tumor from which they develop, the cancer tumours are classified in carcinomas, sarcomas, lymphoma, blastoma and germ cell tumor. Although cell origins are different, they exhibit resembling symptoms and proliferation stages. The first stage of cancer development is usually associated with no visible symptoms, but as the tumor advances to higher stages the patient can manifest ulceration, pain and bleeding. Usually the tumor growth is blocking the circulatory system and organs making them unable to function properly.[57]

I.1.2.4. Organic chemistry in anticancer research

Starting in 1950 many research groups, especially in the chemotherapy field, have undertaken great efforts in order to study the physiology and behaviour of tumor cells and to develop effective anticancer drug-systems. The first approach in the area of anticancer chemotherapy was the successful clinical use of Mustardgen against human lymphoma.[58] This warfare chemical (chlormethine) represents the nitrogen analogue of mustard gas. At the same time, an American pediatric pathologist, Sidney Farber, noticed that folic acid (vitamin B9) played an important role in the proliferation of acute lymphoblastic leukemia. By using

aminopterin and amethopterin (folic acid antagonists) against acute lymphoblastic leukemia cells he observed a tumor regression.[59] Some years later methotrexate successfully treated choriocarcinoma. Because of his accomplishments Farber became known as "father of the anticancer chemotherapy". In the next years several metabolites and their analogues were investigated with repect to their anticancer activity. 6-Mercaptopurine and 5-Fluorouracil proved to have very good anti-cancer properties.[60,61]

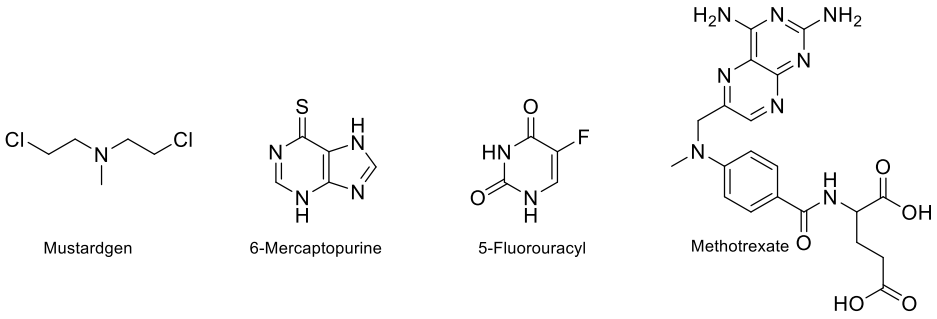


Figure 11. First chemotherapeutic drugs used.

The next step included the screening of a large number of chemical and natural drugs as potential anticancer agents. Starting with 1954 many natural compounds have been used as drugs in anti-cancer therapy. The most eloquent examples include Vincristine (extract from *Vinca Rosea*), Paclitaxel (extract from *Taxus*) and Topotecan (extract from *Camptotheca Acuminata*).[62]

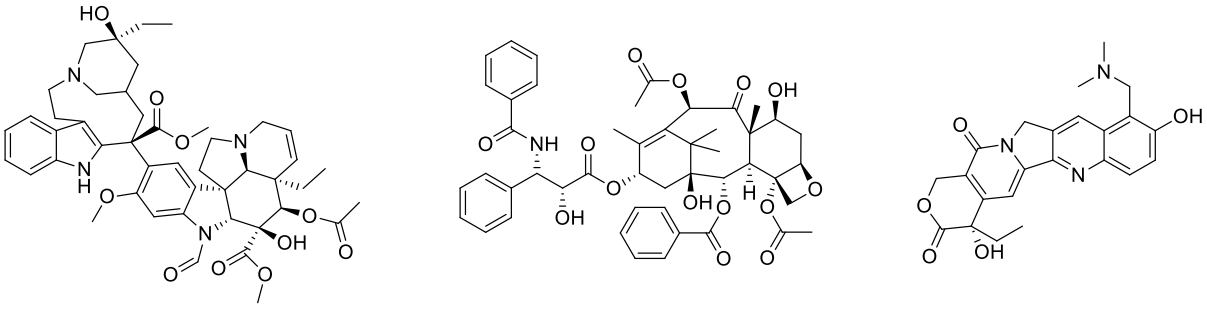


Figure 12. Natural compounds drugs used in anticancer therapy.

Besides searching for active natural product drugs, also the total syntheses of drugs like Fludarabine phosphate (nitrosourea derivate) or Daunorubicine, Doxorubicine (anthracycline compound) were performed (Fig. 13).[63,64] In 1966, Tamoxifen, the first estrogenic receptor antagonist, was synthesized by Dora Richardson.[65,66] After successful clinical trials of Tamoxifen and its analogues and after having established the link between breast cancer and contraception, the FDA (Food and Drugs Administration) approved their use as anticancer agents.

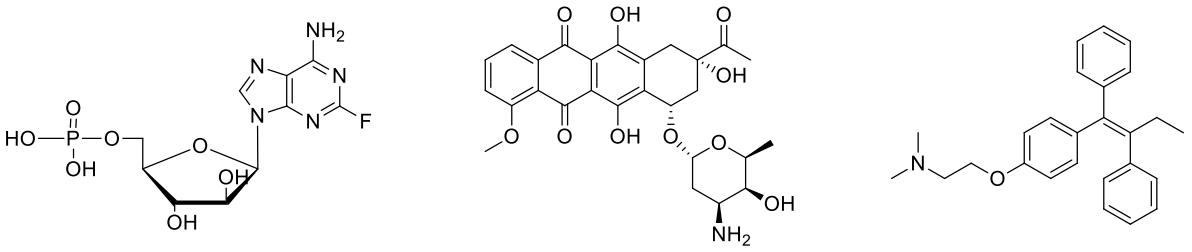


Figure 13. Synthetic compounds drugs used in anticancer therapy.

I.1.2.5. Metal complexes in anticancer research

Compared to chemotherapy with organic compound, the chemotherapy with metal containing drugs has started in the second part of the 19th century. The pioneer compound, potassium arsenite (Fig. 14), was used until 1950 for treatment of a large number of conditions under the name of Fowler´s solution and it also proved to have anti-leukemic effect.[67]

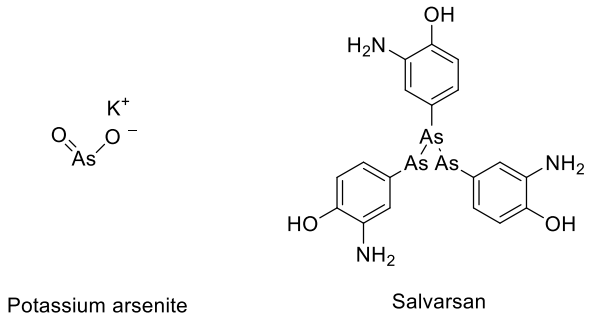


Figure 14. Chemotherapeutic arsenic based drugs.

At the beginning of the 20th century, Salvarsan (Fig. 14), an organoarsenamide derivative, was involved in the treatment of syphilis, a disease caused by the infection with the spirochaete bacterium *Treponema pallidum*.[68] The drug was produced by the German company Hoechst and it became a blockbuster in the world's drug market. However, its days of fame ended around 1940, when penicillin was discovered. Some years later, gold-containing drugs like Aurofin or Aurothiosulphate were reported to exhibit good anti-rheumatic properties (Fig. 15).[69]

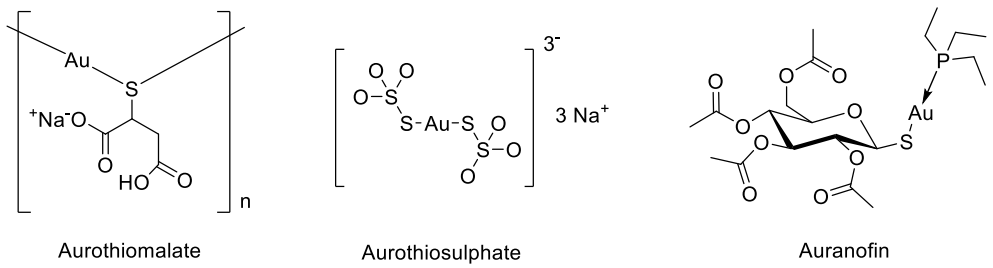


Figure 15. Anti-rheumatic gold containing drugs.

The milestone compound Cisplatin was discovered by Barnett Rosenberg in 1965. He observed the generation of a soluble platinum complex during the electrolysis of platinum electrodes which inhibited binary fission in *Escherichia coli* bacteria.[70] Starting from 1978, Cisplatin is extensively used as anticancer agent against several types of tumours (lung, testicular, ovarian).[71,72] However, the extreme side effects of the Cisplatin therapy (nephrotoxicity, neurotoxicity, nausea, hair loss etc.)[73] has led to the development of other platinum-containing compounds that are more tolerable by the human body, like Carboplatin and Oxaliplatin (Fig. 16).[74]

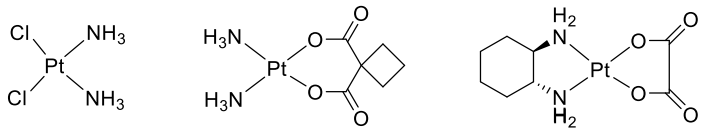


Figure 16. Platinum complexes used as antitumor agents.

Another successful metal in the area of medicinal chemistry is ruthenium. These compounds feature a wide range of bioactivity including strong cytotoxicity, but at the same time they show only mild undesired side effects along with convincing anti-metastatic properties, which make ruthenium complexes the second most studied class of metal complexes after platinum compounds.[75] The best representatives of Ru-containing drugs include KP1019 and NAMI-A which are currently in clinical trials (Fig. 17).[76]

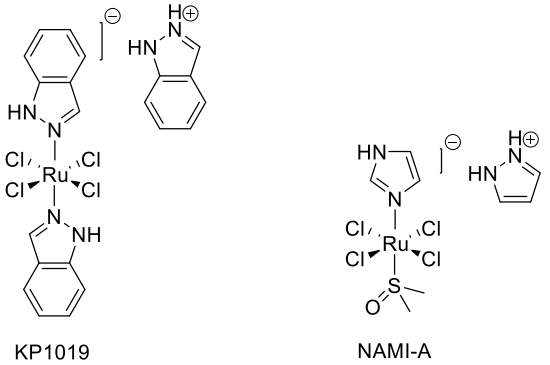


Figure 17. Ruthenium complexes with good antitumor activity.

In some cases, researchers designed the analogous metal complex of already marketed compounds in order to surmount resentence to the benchmark drug (Fig. 18). This approach was successfully used in the case of Tamoxifen and Chloroquine. The metallocenyl derivative of Tamoxifen, namely Ferrocifen, manages to interact with both estrogenic receptors positive and negative.[77] Ferroquine, the metallocenyl complex of Chloroquine, proved to be active against malaria strains which had become resistant to the standard drug Chloroquine.[78]

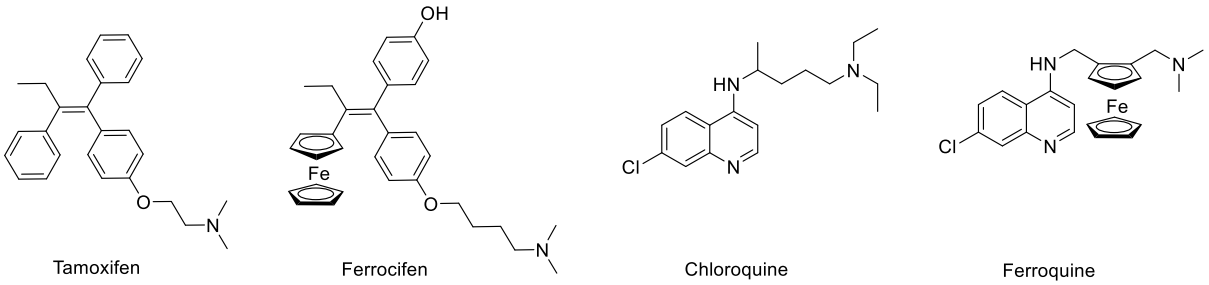


Figure 18. Metallocenyl complexes of Tamoxifen and Chloroquine.

Although the total number of drugs with a metal core actively employed in cancer treatment is not impressive, a large number of metal compounds are actively designed and screened. Many studies are based on carbene chemistry, Theobromine silver derivatives, complexes of Phenantroline with rhodium or iridium etc..[79-82]

I.1.2.6. N-heterocyclic carbenes in anticancer research

The pharmacological applications of NHC metal compounds have only slowly evolved in the last years compared to the rapid growth of the homogeneous transition metal catalysts containg NHC ligands. Although NHC metal complexes readily generated in a simple synthetic approach, their pharmaceutical properties is still underdeveloped.

The design of new NHC metal complexes with various substituents leading to superior antitumor properties represents the highly topical field of chemical research. In the last decades gold-containing derivatives have been employed for the development of novel bioactive drugs including those with anticancer activity. The fact that Au(III) and Pt(II), both d^8 ions, adopt a square planar ligand arrangement along with the immuno-modulatory properties of the gold(I)-containing anti-rheumatic compounds and the insertion of gold (I and III) in the molecule of antitumoral agents, represent the main arguments for the design and screening of new gold-containing drugs for anticancer activity.[83]

Although Auranofin and its analogues opened the path of gold containing complexes with applications in chemotherapy, some areas of activity remains still open for studies.[84] In order to fill this gap, many different ligands (NHCs, porphyrines, phosphines, dithiocarbamates etc.) have been introduced in the coordination gold sphere over the last years in order to develop new bioactive molecules.[85-88]

Lipophilicity of mono-NHC-gold(I) complexes, known as mitochondria targeting complexes, play an important role in achieving the desired biological activity.[89] Using this hypothesis, many research groups dedicated their work to improve the biological properties of Au–NHC complexes by tuning the ligand lipophilicity.

Weaver *et al.* reported the anti-cancer activity for a series of NHC-Au(I) complexes against prostate, bladder and breast tumor cell lines.[90] The best results were obtained for two compounds (Figure 19), with an IC_{50} value of 0.18 μM against a bladder tumor cell line and 0.9-1.9 μM for other two tumor cell lines.

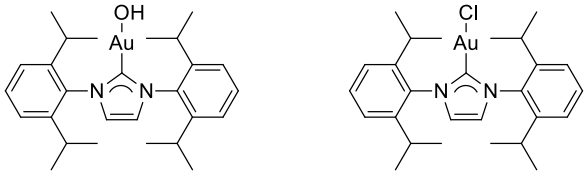


Figure 19. NHC-Au(I) complexes reported by Weaver *et al.*.

Nevertheless, when their cytotoxicity was compared to a human breast carcinoma cell line and a normal breast epithelial cell line, they, unfortunately, showed no selectivity.

Ott *et al.* explored a series of benzimidazole-NHC gold(I) complexes as TrxR and GR (glutathione reductase) inhibitors (Figure 20) and their results are consistent with a selective inhibition of TrxR over GR. Furthermore all gold compounds showed significant antiproliferative effect with IC_{50} values in the low micromolar range.[91]

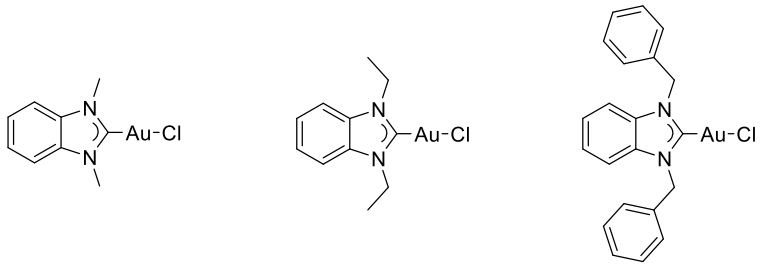


Figure 20. NHC-Au(I) complexes reported by Ott *et al.*.

Using the silver transmetallation route, Patil *et al*. manage to synthesize and evaluate the biological activity of cyanobenzyl and benzyl NHC-Au(I) complexes (Figure 21), which showed only moderate activity against human cancerous renal cell line Caki-1.[92]

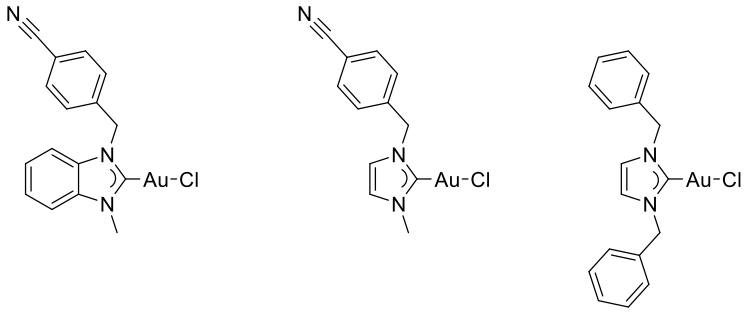


Figure 21. NHC-Au(I) complexes reported by Patil *et al.*

An interesting series of diarylimidazolin-2-ylidene gold(I) NHC complexes were prepared and evaluated for their biological activity by Liu *et al.*. Some of this complexes revealed increased growth inhibitory effects against MCF-7, MDA-MB 231 and HT-29 (Fig. 22).[93,94]

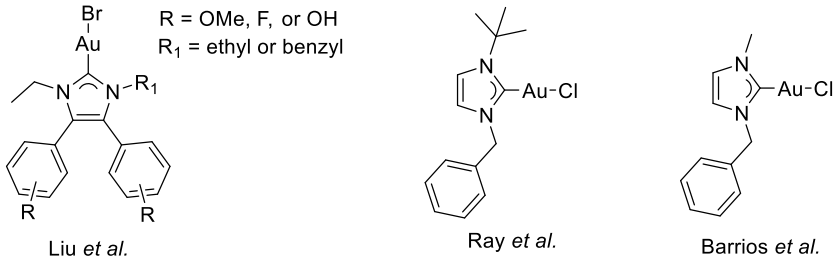


Figure 22. NHC-Au(I) complexes reported by Liu *et al.*, Ray *et al.* and Barrios *et al.*.

Furthermore Ray *et al.* studied the antiproliferative properties of some NHC-Au(I) complexes against HeLa cells, but only low growth inhibitory effect were found.[95] Similar *in vitro* results were conducted by Barrios *et al.* on other compound against PEST (peptide sequence that is rich in proline (P), glutamic acid (E), serine (S), and threonine (T)).[96]

I.1.2.7. Lipinski's Rule of Five

After the physical-chemical investigation of a huge number of drugs and possible drugs already in clinical testing, Christopher Lipinski at al.[97-99] managed to establish a guiding principle for the prognosis of compound absorption through the cell membrane. The main remark from the medicinal chemist was that the majority of the drugs used are rather small, lipophilic molecules. The rule was elaborated in 1997 and stated that the membrane permeability and body absorption is favourable when the molecules have the following properties:

- The molecular weight is smaller than 500g/mol.
- The sum of hydroxyl and amine groups (hydrogen bond donors) is less than 5.
- The sum of nitrogen and oxygen atoms (hydrogen bond acceptors) is less than 10.
- The lipophilicity value of the molecule is less than 5.

However, natural products and the substrates used for transporters do not follow this rule. Although the rule is very valuable and intensively used, it has also some limitations. An analysis of cca. 60 of the pharmaceutic blockbuster drugs from 2007 under the criteria of *Lipinski´s rule of five*, revealed that 7 of them (Docetaxel, Montelukast, Atorvastatin, Telmisartan, Leuprolide, Tacrolimus and Olmesartan) did not match the rule parameters and 5 of them missed just one of the requested criteria.[100]

Nevertheless, in order to synthesise, screen and evaluate in short time period a large number of compounds with a high probability of becoming a hit-molecule in medicinal chemistry, *the Lipinski´s Rule of five* provides an excellent starting point.

I.2. 1,2,4-Oxadiazoles

I.2.1. Chemical reactivity of 1,2,4-oxadiazoles

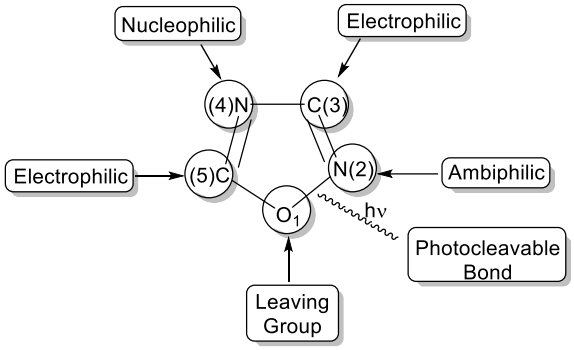


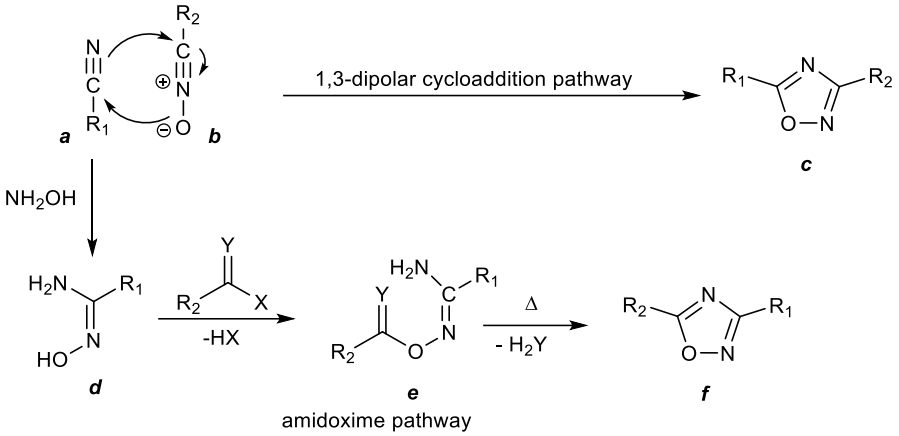
Figure 23. Multifunctional features of 1,2,4-oxadiazole ring.

1,2,4-Oxadiazole has an aromaticity index of I_A = 48. This feature makes them one of the weakest aromatic systems within the class of five-membered heterocycles,[101,102] which results in an increased tendency to rearrange into more stable systems. The molecular rearrangements of the 1,2,4-oxadiazole ring can be traced to the labile O-N bond by employing several thermal or photochemical procedures, and this property can be used as an alternative route in the synthesis of several other heterocycles.[103]

The electrophilic character of C(3) and C(5)[104-107] allows the generation of compounds which are usually very difficult to prepare. One example includes the irreversible ring-degeneration rearrangement taking place on 1,2,4-oxadiazole when it is attacked by an external bidentate nucleophile.[108] This property is further enhanced in the presence of electron-withdrawing substituents. The pyridine-like nitrogen N(4) present in the 1,2,4-oxadiazole ring may act as a nucleophile[109] and makes the molecule slightly basic.[110] On the other hand the N(2) nitrogen has an ambiphilic character.[111] Another important attribute of the 1,2,4-oxadiazole ring is the capability of the oxygen atom of the ring moitey acting as an internal leaving-group.[112]

All these properties suggest that the 1,2,4-oxadiazole ring is a versatile heterocycle. The reactivity is strongly correlated with the nature of the substituents and the reaction conditions involved.

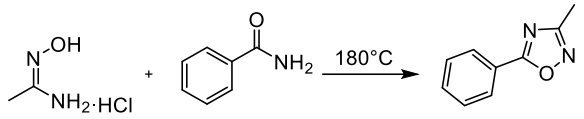
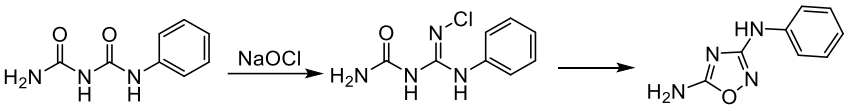
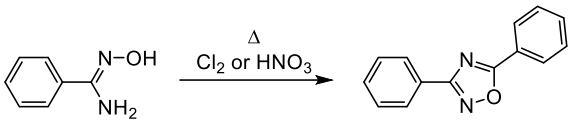
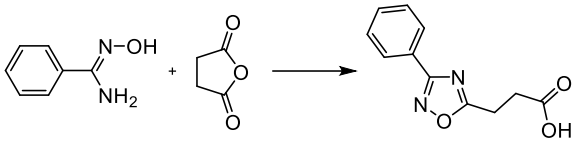
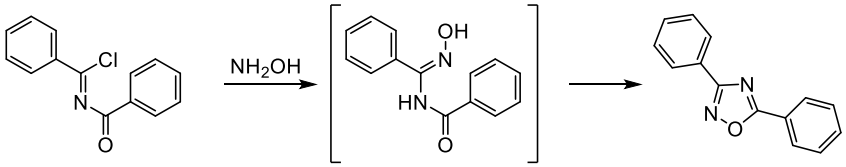
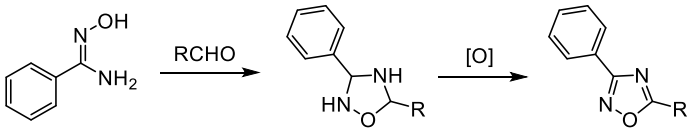
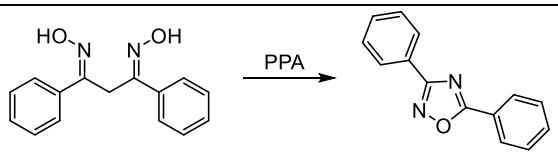
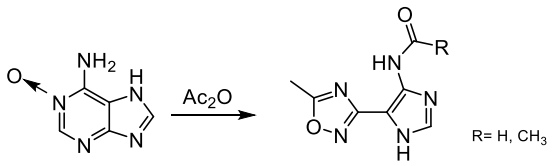
I.2.2. Synthetic aspect of 1,2,4-oxadiazoles



Scheme 1. General synthetic pathways regarding 1,2,4-oxadiazoles.

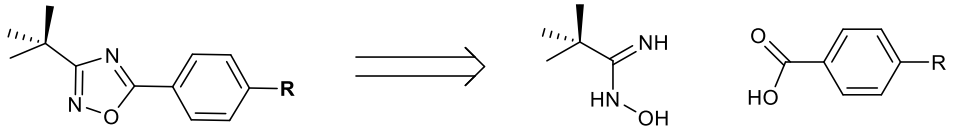
"Give me a nitrile and I will build you a 1,2,4-oxadiazole!"[113] This affirmation made by Pace *et al.* condenses the two most frequently employed synthetic pathways in the preparation of substituted 1,2,4-oxadiazoles[30,114-118] (Scheme 1): The 1,3-dipolar cycloaddition of nitriles ***a*** with nitrile oxides ***b***; the cyclodehydration of amidoxime derivatives ***e***. The amidoximes can be readily generated from nitriles ***a*** and hydroxylamine, NH_2OH, followed by coupling with activated carboxylic acids or their derivatives including amidoxime itself. The main benefit of these synthetic routes is their complementarity. The nitrile precursor substituent (R_1 in the scheme 1) can be implemented at either C(5), as in ***c***, or C(3), as in ***f***, of the final 1,2,4-oxadiazole. However, the limitation of these methods lies with the accessibility of the precursors.

Tabel 2. Synthetic pathways for 1,2,4-oxadiazoles.

Cyclisation of amidoximes with amides	 1,2,4-Oxadiazoles can be synthesized from the corresponding amide by reacting an amidoxime with an amide at elevated temperatures (160-180°C).
Oxidation of imino group	 The guanidine derivative can be successfully oxidized with sodium hypochlorite in order to obtain the 1,2,4-oxadiazole.[119]
Pyrolyses of amidoximes and their esters	 Amidoximes at 170°C, in the presence of fatty acids with low molecular weight,[120] nitrous acid or chlorine[121] produce 1,2,4-oxadiazole.
Starting from benzamidoximes	 By reacting benzamidoximes derivatives with succinic, acetic, propionic or butyric anhydride it is generated the corresponding 1,2,4-oxadiazoles.[122]
Ring closure of monoximes of diacylamides	 The imidyl chloride of dibenzamide can be reacted with hydroxylamine in order to form 1,2,4-oxadiazole.[123]
Oxidation of 1,2,4-oxadiazolines	 Aromatic amidoximes can be reacted with aldehydes to give as condensation products the corresponding dihydro-1,2,4-oxadiazoles.[124,125]
Dehydration with rearrangement of glyoximes	 The reaction of *α*-benzildioxime with polyphosphoric acid (PPA) at 120°C generates exclusively 1,2,4-oxadiazole.[126]
Ring transformations	 When refluxed in acetic anhydride, adenine 3-*N*-oxide is fast converted into 4-formamido and 4-acetamido-5-(5-methyl-1,2,4-oxadizol-3-yl)imidazole.

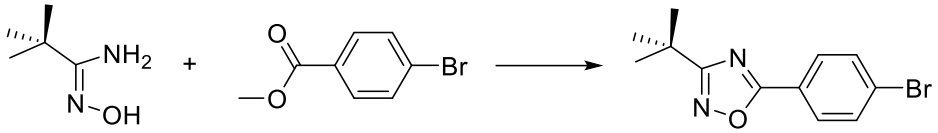
I.3. Motivation

1,2,4-Oxadiazole heterocycles represent an interesting biological functionality since they are bioisosteres of esters and amides.[31,127,128] Furthermore these compounds seem to have a much better hydrolytic and metabolic stability.



Scheme 2. Retrosynthetic scheme for 3,5-substituted 1,2,4-oxadiazole system.

In an attempt to produce new pyrroline-based insecticides, Bayer CropScience reported the preparation of 5-(4-bromophenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole[129] by an cyclisation reaction between *tert*-butyl amidoxime and the methyl ester of *p*-bromo benzoic acid (Scheme 3).



Scheeme 3. Reported synthesis of 3-(*tert*-butyl)-5-(phenyl)-Br substituted derivative.

The 1,2,4-oxadiazole ring is 3-substituted with an *tert*-butyl group which confers stability to the ring whereas the bromophenyl substituent in 5-position allows for further reactivity. This is the only reported example of an 3-(*tert*-butyl)-5-(phenyl)-substituted 1,2,4-oxadiazole and it was employed as a coupling reagent in order to produce the bioactive insecticide agent 3-(*tert*-butyl)-5-(4'-(2-(2,6-difluorophenyl)-3,4-dihydro-2H-pyrrol-5-yl)-3'-fluoro-[1,1'-biphen-yl]-4-yl)-1,2,4-oxadiazole (Figure 24).

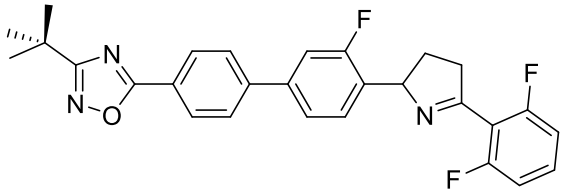


Figure 24. Reported 3-(*tert*-butyl)-5-(phenyl)-substituted insecticide derivative.

Taking these aspects into consideration, the strategy was to employ this molecular fragment to build a library of possible bioactive compounds.

II. Objectives

Aim of this dissertation was the synthesis, characterisation and antitumor evaluation of distinct 1,2,4-oxadiazole-containing compounds and their corresponding NHC-Au(I) complexes (Figure 25).

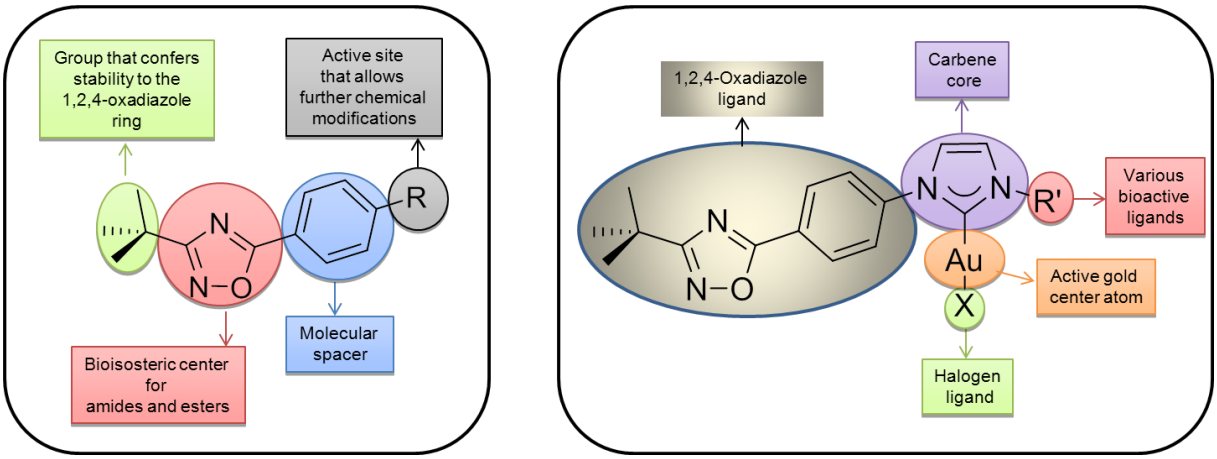


Figure 25. Graphic illustration of the target compounds.

The implementation of the 1,2,4-oxadiazole unit was chosen for its known ability of acting as a bioisoster for amides, carbamates, esters and hydroxamic esters. The N-heterocyclic carbene complexes (Arduengo-type) bearing on one side the 1,2,4-oxadiazole heterocycle has been assigned as a second key ligand in the development of novel compounds with antitumor properties. The metal of choice was gold(I), known for providing coordinative stability and good antitumor activity. Appropriate tuning of the NHC core by inserting other bioactive ligands should furnish distinguished biological activity. In addition to gold (I), other metal centers were created (silver, rhodium, ruthenium and palladium) and tested for their antitumor activity. A professional antitumor evaluation will determine the potential of the target compounds as anticancer agents.

The antitumor activity of these compounds will be tested in a monolayer cell survival and proliferation assay using human tumor cell lines of different origin/histotype which allow for the analysis of their potency and tumor selectivity. Central theme of this PhD dissertation is to gather sufficient synthetic and biological information to establish a very versatile synthetic methodology for the preparation of 1,2,4-oxadiazole related compounds and to tune their application as as antitumoral agents.

III. Results and discussion. 1,2,4-Oxadiazole containing ligands

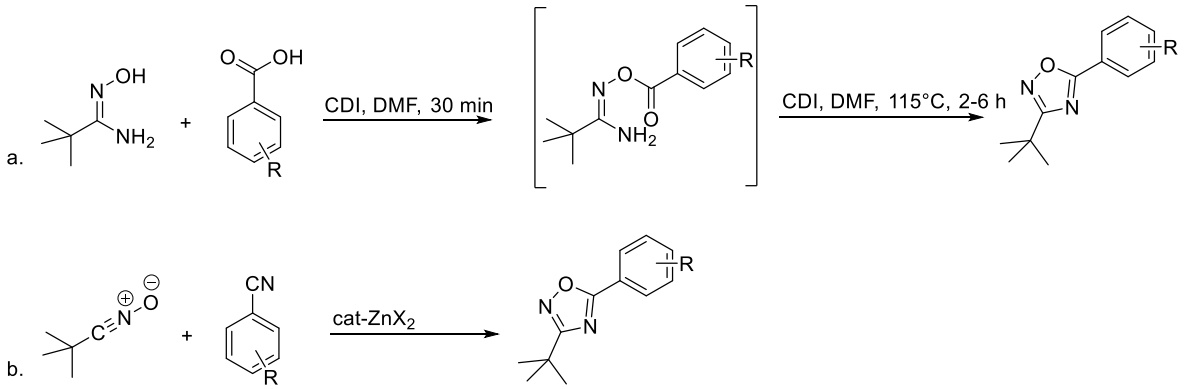
III.1. Introduction

Clapp reviewed the synthesis of 1,2,4-oxadiazoles[118] and pointed out that two general methods dominate their preparation (~95%):

a) The condensation of amidoximes with carboxylic acid derivatives.

b) The dipolar cycloaddition of nitrile oxides to nitriles.

The general approach for the synthesis of 1,2,4-oxadiazoles is illustrated in Scheme 4.



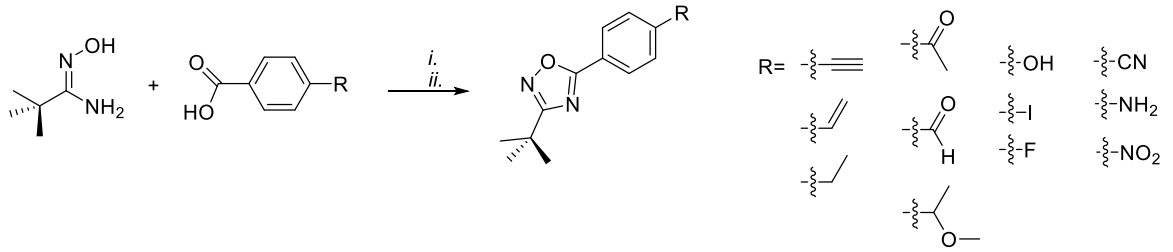
Scheme 4. Common synthetic strategies towards 1,2,4-oxadiazoles; a. amidoxime route; b. 1,3 dipolar cycloaddition route

Using route ***a***, the amidoxime route, the carboxylic acid has to be employed in an activated form. The activated carboxylic acid can be prepared beforehand or *in situ* by several methods,[130] e.g. as an acyl chloride or by the use of *N*,*N*′-carbonyldiimidazole (CDI). In the first step the amidoxime is *O*-acylated with the activated derivative in a condensation reaction. The *O*-acylated amidoxime can either be isolated or immediately undergo the cyclisation to the heterocyclic oxadiazole ring. This cyclodehydration reaction takes place upon heating to temperatures above 115°C.[131,132] Microwave techniques have also been employed in the synthesis of these heterocycles, but the advantage of CDI is that it activates the carboxylic acid *in situ* and can be used for step 1 and step 2 in DMF.

III.2. One step synthesis of new members belonging to 1,2,4-oxadiazole family

Scheme 5 presents the one-pot synthesis of 1,2,4-oxadiazols starting from *tert*-butyl-amidoxime and 4-substituted-benzoic acids. Activation of the 4-substituted-benzoic acid with CDI and further acylation of the *tert*-butyl-amidoxime in DMF as solvent furnished the *O*-

acylamidoxime, which was not isolated; on heating to 120°C for several hours, it underwent a cyclodehydration reaction, delivering the cyclized products in good to moderate yields after purification.

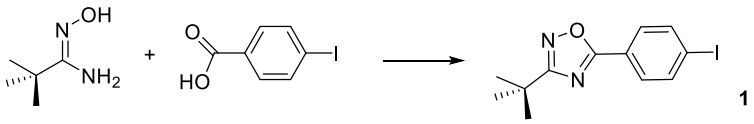


Scheme 5. One-pot synthesis of 1,2,4-oxadiazols using the amidoxime route; *i*. 1,1 eq. CDI in DMF, 30 minutes; *ii*. 1,1 eq. CDI in DMF, 120°C, 4h.

III.2.1. One step synthesis of halogen containing 1,2,4-oxadiazoles.

III.2.1.1. Synthesis of 3-(*tert*-butyl)-5-(4-iodophenyl)-1,2,4-oxadiazole (1)

One of the first molecule obtained, 3-(*tert*-butyl)-5-(4-iodophenyl)-1,2,4-oxadiazole (**1**), belongs to this class of compounds. Its synthesis is depicted in scheme 6.



Scheme 6. Synthesis of 3-(*tert*-butyl)-5-(4-iodophenyl)-1,2,4-oxadiazole (**1**).

Using 4-iodobenzoic acid as starting material the new 1,2,4-oxadiazole derivative was generated in good yields (85%) and fully characterized. The ^{1}H NMR spectrum shows the expected number of resonances, a multiplet belonging to the four aromatic protons at 7.96-7.79 ppm and the nine protons of the *t*-butyl group at 1.42 ppm as a singlet. The mass spectrometry gives the molecular peak signal M^{+} 328.0 along with other fragments. Crystals suitable for X-ray diffraction analysis were obtained by slow diffusion of hexane in a concentrated solution of **1** in DCM.

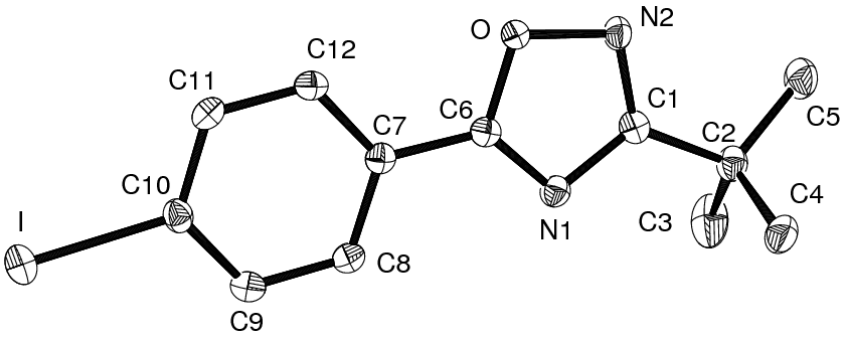
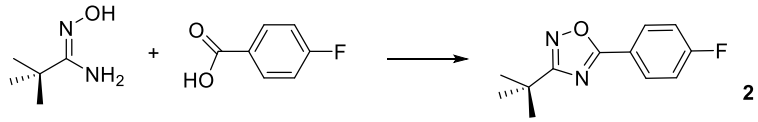


Figure 26. Molecular structure of 3-(*tert*-butyl)-5-(4-iodophenyl)-1,2,4-oxadiazole (**1**). Atoms are drawn as 50% thermal ellipsoids. Selected bond lengths [Å] and angles [°]: C1-N1 1.380(2), C1-N2 1.305(2), N2-O 1.4228(16), C6-O 1.3434(18), C6-N1 1.299(2), C10-I 2.0965(16), N1-C1-N2 115.04(14), C11-C10-I 120.60(11).

The molecular structure of **1** was established by X-ray diffraction analysis and shown in Figure 26. The compound **1** crystallizes in the monoclinic space group $P2_1/c$. The planar molecule features a C10-I bond length of 2.0965(16) Å and the angle C11-C10-I is 120.60(11)°.

III.2.1.2. Synthesis of 3-(*tert*-butyl)-5-(4-fluorophenyl)-1,2,4-oxadiazole (2)

4-Fluorobenzoic acid was used as starting material in order to prepare 3-(*tert*-butyl)-5-(4-fluorophenyl)-1,2,4-oxadiazole (**2**) in moderate yields (45%) as shown in Scheme 7. The low yield for this one-pot cyclisation is explained by the formation of the imidazole substituted 1,2,4-oxadiazole (**30**) as a secondary product.



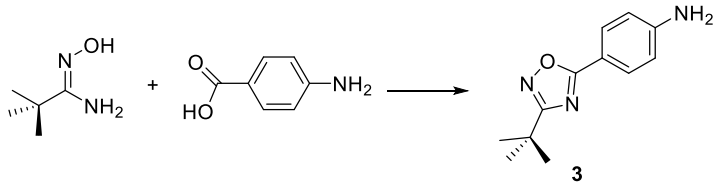
Scheme 7. Synthesis of 3-(*tert*-butyl)-5-(4-fluorophenyl)-1,2,4-oxadiazole (**2**).

The m/z indicates the molecular peak at M^+ = 220.1, along with characteristic fragments at M^+-15 and M^+-97. The ^{1}H-NMR spectrum of **2** recorded in $CDCl_3$ gives the coresponding signals attributed to the four protons in the aromatic area along with the *t*-Bu signal at 1.43 ppm.

III.2.2. One step synthesis of amino containing 1,2,4-oxadiazoles.

III.2.2.1. Synthesis of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)aniline (3)

Scheme 8 presents the one-pot synthesis of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)aniline (**3**) starting from *tert*-butyl-amidoxime and 4-aminobenzoic acid. Activation of the 4-aminobenzoic acid with CDI and further acylation of the *tert*-butyl-amidoxime in DMF as solvent furnished the *O*-acylamidoxime, which was not isolated. Heating to 120°C for four hours induced the cyclodehydration reaction to yield aniline **3** in 59% yield after purification.



Scheme 8. One-pot synthesis of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)aniline (**3**) using the amidoxime route.

The structure of compound **3** was confirmed by X-ray structure determination (Figs. 27 and 27a). It crystallizes with two molecules in the asymmetric unit, which differ in the relative orientation of the rings (interplanar angles 22 and 9°). Three of the four NH hydrogens are involved in hydrogen bonds, leading to ribbons of H-bonded rings parallel to the *a* axis.

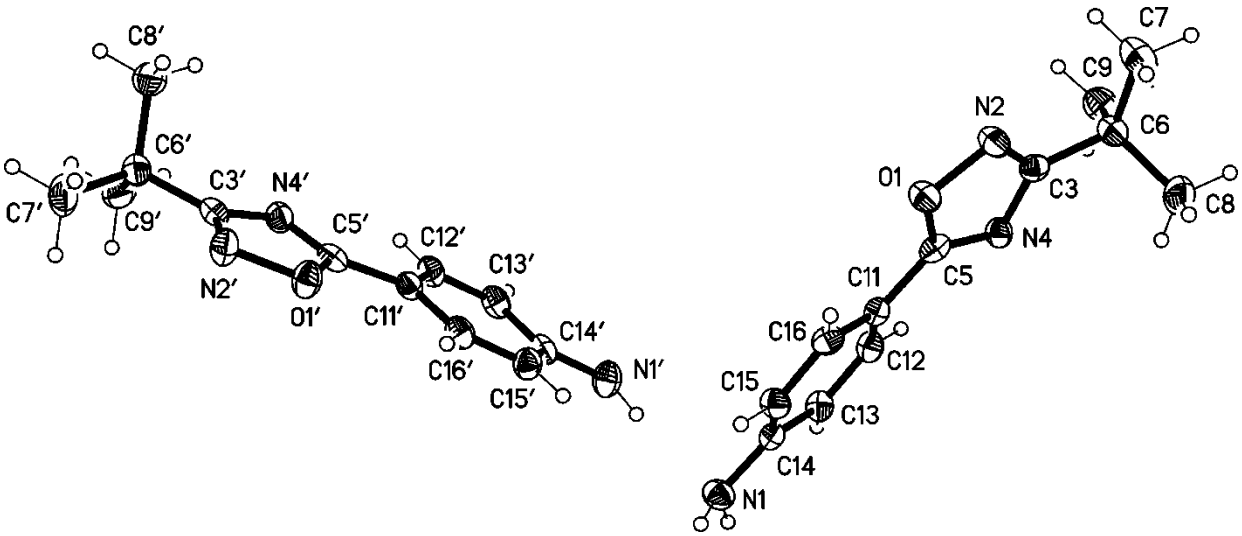


Figure 27. Molecular structure of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)aniline (**3**). Atoms are drawn as 50% thermal ellipsoids. One hydrogen at N1' is eclipsed.

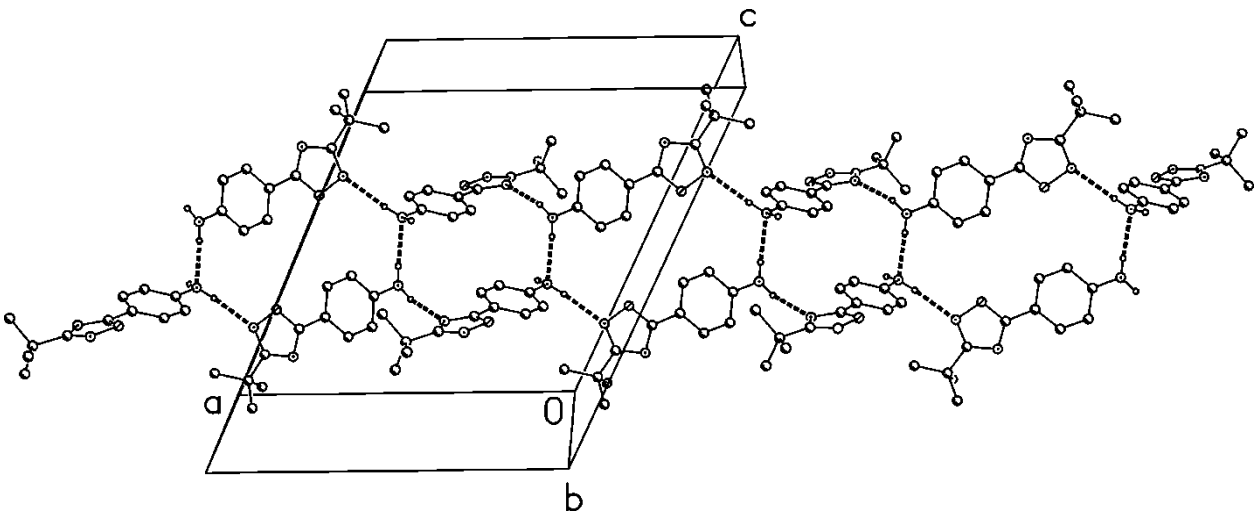


Figure 27a. Packing diagram of compound **3**. Hydrogen bonds are indicated as dashed lines.

Following the second synthetic route, the 1,3-dipolar cycloaddition, with the purpose of increasing the yield of compound **3**, we used *p*-toluenesulfonic acid (PTSA)-$ZnCl_2$ as a catalyst for the synthesis of aniline **3** from amidoximes and organic nitriles.[133] *Tert*-butyl-amidoxime and 4-aminobenzonitrile were mixed in DMF with catalytic amounts of PTSA-$ZnCl_2$. First, *tert*-butyl-amidoxime is activated by PTSA-$ZnCl_2$. This results in the formation of a Lewis acid-ammonia complex as a leaving group forming the the nitrile oxide. The 1,2,4-oxadiazole moiety is established by the 1,3-dipolar cycloaddition of nitrile oxide to the 4-aminobenzonitrile. However, the Lewis acid might also be involved in the formation of the heterocycle via a Lewis acid catalyzed [3+2] cycloaddition reaction. Unfortunately, the yield for this reaction was only very low (<20%).

III.2.2.2. Synthesis of 3-*tert*-butyl-5-(4-nitrophenyl)-1,2,4-oxadiazole (4)

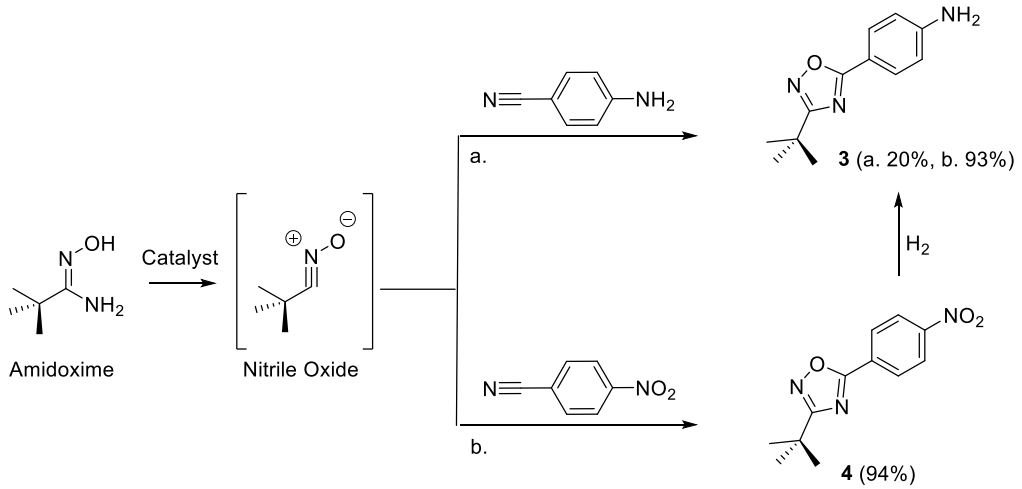
In order to obtain compound **3** via the 1,3-dipolar cycloaddition route we changed the protocol. We synthesized *in situ* the nitro derivative **3** (3-*tert*-butyl-5-(4-nitrophenyl)-1,2,4-oxadiazole) using the same catalyst pair as described above. The hydrogenation of the nitro compound **4** to the corresponding amine **3** was conducted in the same reaction vessel without isolation of the intermediate. The overall yield in this case was 64%. Intermediate **4** was also isolated in one case for its full characterisation. After a series of test reactions using various acids as catalyst (*p*-toluensulfonic acid-PTSA, 2-mesitylenesulfonic acid-MSA and methanesulfonic acid-MeSA) in combination with $ZnCl_2$ and $ZnBr_2$, MSA-$ZnBr_2$ in acetonitrile proved to be the best combination to achieve a facile preparation of compound **4** from *tert*-butyl-amidoxime and 4-nitrobenzonitrile under mild conditions. The results of the

screening process are summarized in Table 1. The optimized yield was 93% which makes this route far more practical than the amidoxime route presented in Scheme 9 for which the yield was just 59%.

Table 3. Optimal conditions for the 1,3 dipolar cycloaddition route.[a)]

Entry	Catalyst 1	Catalyst 2	Solvent	Time (h)	Yield %
1	PTSA	$ZnCl_2$	DMF	5	64
2	MSA	$ZnCl_2$	DMF	3	76
3	MeSA	$ZnCl_2$	DMF	12	56
4	PTSA	$ZnBr_2$	DMF	5	70
5	MSA	$ZnBr_2$	DMF	3	79
6	MeSA	$ZnBr_2$	DMF	12	58
7	PTSA	$ZnCl_2$	MeCN	2	82
8	MSA	$ZnCl_2$	MeCN	2	92
9	MeSA	$ZnCl_2$	MeCN	12	63
10	PTSA	$ZnBr_2$	MeCN	2	86
11	MSA	$ZnBr_2$	MeCN	2	93
12	MeSA	$ZnBr_2$	MeCN	12	65

a) General conditions: *tert*-butylamidoxime (1 eq), 4-nitrobenzonitrile (1 eq), catalyst 1 (0.3 eq), catalyst 2 (0.3 eq), 80ºC.



Scheme 9. One-pot synthesis of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)aniline (**3**) using the 1,3 dipolar cycloaddition of amidoxime to the nitrile oxide route.

The molecular structure of compound **4** was confirmed by X-ray structure determination (Figs. 28 and 28a). The interplanar angle in compound **4** is only 3(16)°and the molecules are linked to ribbons parallel to the *b* axis by two C–HΛO interactions.

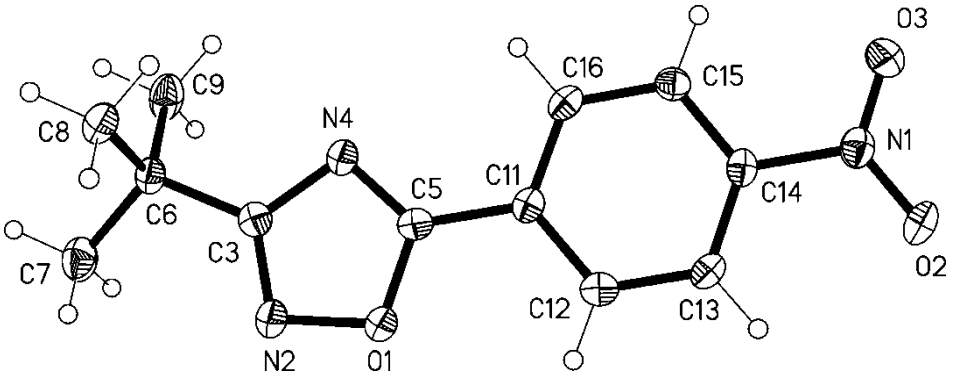


Figure 28. Molecular structure of 3-*tert*-butyl-5-(4-nitrophenyl)-1,2,4-oxadiazole (**4**). Atoms are drawn as 50% thermal ellipsoids.

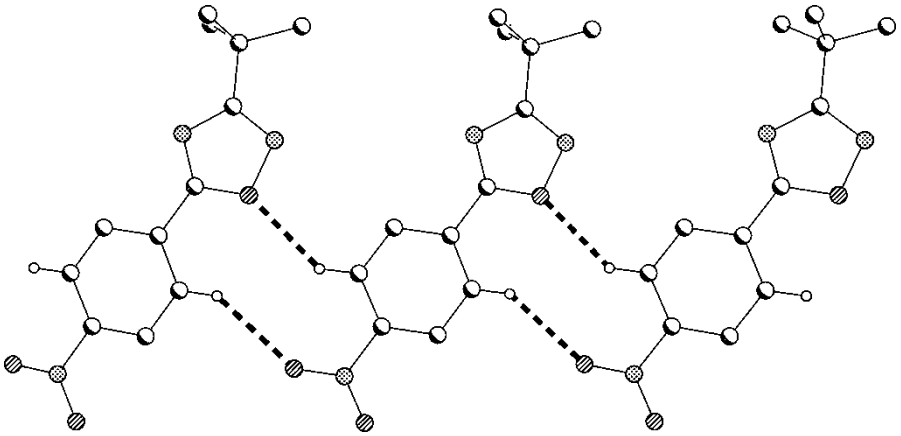
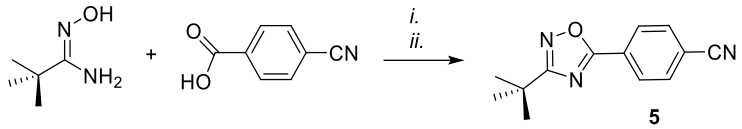


Figure 28a. Packing diagram of compound **4** showing C–HΛO interactions.

III.2.3. One step synthesis of 1,2,4-oxadiazoles bearing heteroatoms.

III.2.3.1. Synthesis of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzonitrile (5)

As a precursor for several 1,2,4-oxadiazol derivatives, 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzonitrile (**5**) was generated from commercially available 4-cyanobenzoic acid and *tert*-butyl-amidoxime (Scheme 10). Activation of the 4-cyanobenzoic acid with CDI (1,1'-carbonyldiimidazole) and further acylation of the *tert*-butyl-amidoxime in DMF as solvent furnished the *O*-acylamidoxime, which was not isolated. On heating to 120°C for several hours, it underwent cyclisation with the elimination of one molecule of water, delivering the nitrile **5** in 76% yield after purification.



Scheme 10. One pot synthesis of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzonitrile (**5**) using the amidoxime route; *i*. 1,1 eq. CDI in DMF, 30 minutes; *ii*. 1,1 eq. CDI in DMF, 120°C, 4h.

Compound **5** was fully characterized. The ^{1}H NMR data in $CDCl_3$ reveal the aromatic protons at 8.25 and 7.80 ppm along with nine protons of the *t*-butyl group at 1.43 ppm as a sharp singlet. The MS data include the molecular peak m/z = 227.1 (M^+) and two main characteristic defragmentation fragments (M^+ - 15, M^+ - 97). The solid-state structure of **5** was established by X-ray diffraction analysis (Figure 29).

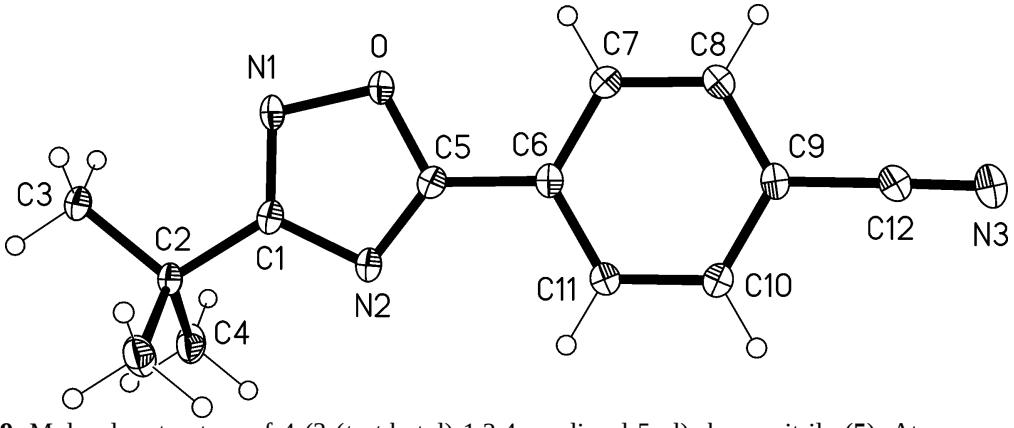
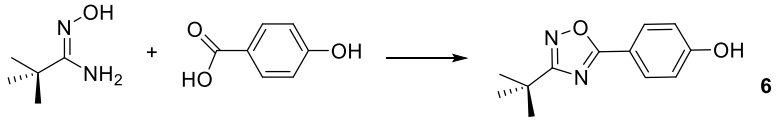


Figure 29. Molecular structure of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)- benzonitrile (**5**). Atoms are drawn as 50% thermal ellipsoids. Selected bond lengths [Å] and angles [°]: C1-N1 1.3074(17), C1-N2 1.3871(15), N1-O1 1.4163(14), C5-O 1.3437(15), C5-N2 1.3013(17), C12-N3 1.1438(18), C9-C12-N3 179.16(14).

Slow evaporation in open atmosphere of a concentrated solution of **5** in DCM gave colorless crystals suitable for single X-ray diffraction measurement. The molecule crystallizes with imposed mirror symmetry in the monoclinic space group $P2_1/m$, whereby only the methyl group at C4 and two hydrogens of the methyl group at C3 lie outside the mirror plane.

III.2.3.2. Synthesis of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenol (6)

The reaction of 4-hydroxybenzoic acid with (*Z*)-*N*'-hydroxypivalimidamide under the general condition presented for the cyclisation formed 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenol (**6**) as a colorless solid in moderate yield (64%).



Scheme 11. Synthesis of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenol (**6**).

The ^{1}H NMR specrum recorded in DMSO-d_6 features one broad resonance corresponding at 10.45 ppm which belongs to the OH group, the resonances in the aromatic region integrated to 4 protons and the *t*-butyl signal appears at 1.33 ppm. The m/z shows the M^{+}= 218.2 and two main fragments (M^{+}-15 and M^{+}-97) characteristic for these molecules (Figure 30).

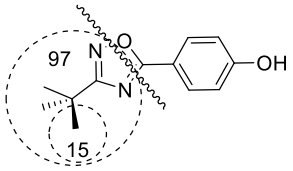
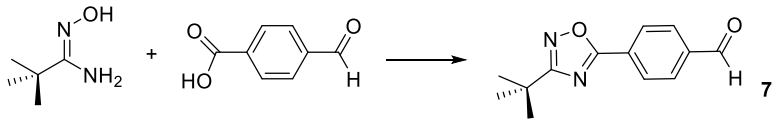


Figure 30. Fragmentation pattern of 3-(*tert*-butyl)-1,2,4-oxadiazol compounds.

III.2.3.3. Synthesis of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzaldehyde (7)

The synthesis of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzaldehyde (**7**) is shown in scheme 12. The acid of choice in this case was 4-formylbenzoic acid. Compound **7** was characterized by NMR spectroscopy and MS analysis (230.1 M^{+}). The aldehyde proton, present at 10.11 ppm in the ^{1}H NMR, along with of the *C*-carbonyl at 191,28 ppm in the ^{13}C NMR spectroscopy clearly proves the structure of the new compound. Crystals suitable for X-ray diffraction analysis were obtained by slow evaporation from a concentrated solution of **7** in DCM.



Scheme 12. Synthesis of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzaldehyde (**7**).

The solid-state molecular structure of **7** was established by X-ray diffraction analysis and shown in Figure 31. The compound crystallizes in the monoclinic space group $P2_1/m$ with two molecules in the unit cell. The planar molecule present a C12-O2 double bond (1.2039(14) Å), with the angle C9-C12-O2 of 124.87(11)°. In the five-membered ring, the bond lengths C1-N1 is 1.3099(14) Å, considerably shorter than the C1-N2 bond 1.3847(12) Å and therefore consistent with a C1-N1 double bond.

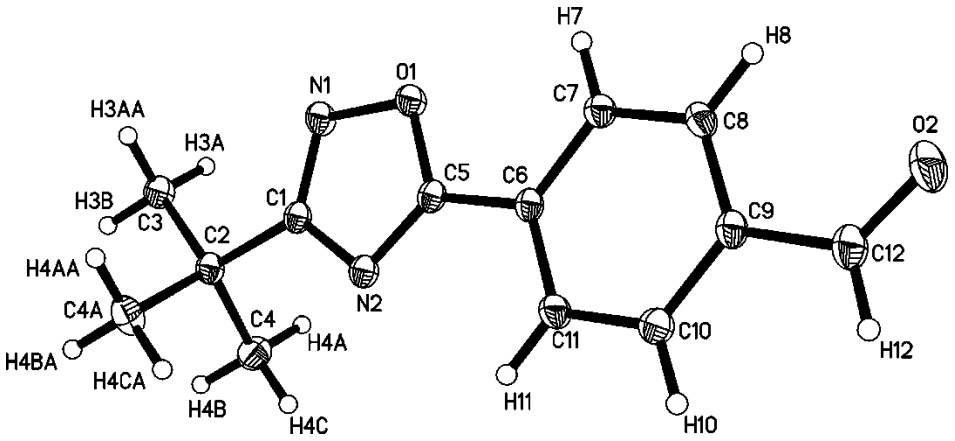
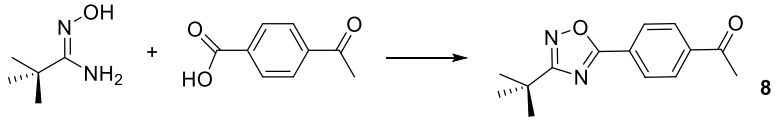


Figure 31. Molecular structure of 4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)benzaldehyde (**7**). Atoms are drawn as 50% thermal ellipsoids. Selected bond lengths [Å] and angles [°]: C1-N1 1.3099(14), C1-N2 1.3847(12), N1-O1 1.4217(11), C5-O1 1.3455(12), C5-N2 1.3006(13), C12-O2 1.2039(14), C9-C12-O2 124.87(11).

III.2.3.4. Synthesis of 1-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)ethan-1-one (8)

Starting from commercially available 4-acetylbenzoic acid we produced in very good yield pure 1-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)ethan-1-one as a colorless solid (Scheme 13). The MS analytics shows the M^+ signal at m/z = 244.1 followed by two main fragments that correspond to the loose of a methyl group (M^+ - 15) and the t-butyl group with the opening of the oxadiazole ring (M^+ - 97).



Scheme 13. Synthesis of 1-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)ethan-1-one (**8**).

The ^{1}H NMR spectrum shows the expected number of signals: The multiplets at 8.25 and 8.10 ppm belong to the four aromatic protons, the resonances at 2.67 and 1.35 ppm correspond to the the methyl and *t*-butyl groups, respectively. The solid-state molecular structure of **8** was established by X-ray diffraction analysis and shown in Figure 32.

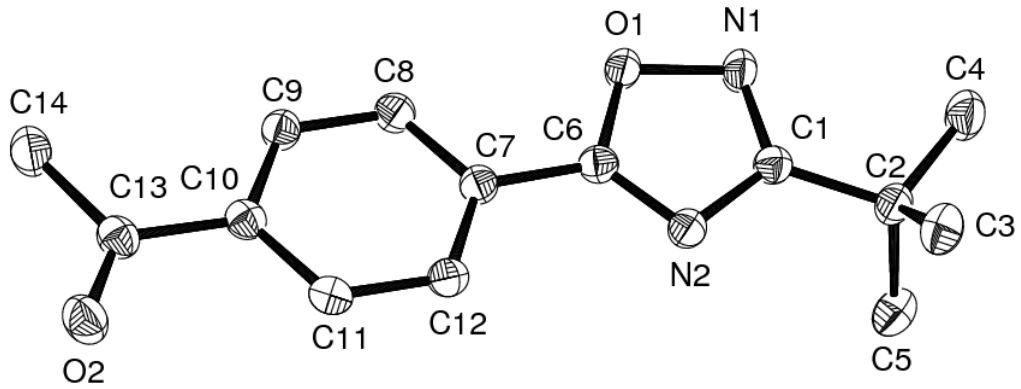
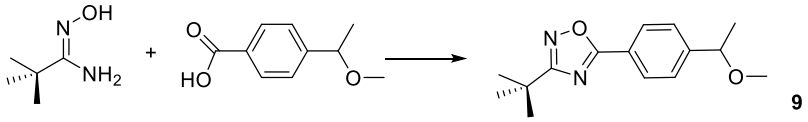


Figure 32. Molecular structure of 1-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)ethan-1-one (**8**). Atoms are drawn as 50% thermal ellipsoids. Selected bond lengths [Å] and angles [°]: C1-N1 1.3061(15), C1-N2 1.3819(14), N1-O1 1.4193(12), C6-O1 1.3418(14), C6-N2 1.2993(14), C13-O2 1.2172(14), C10-C13-O2 120.92(10).

The compound **8** crystallizes in the triclinic space group *P*-1 with two molecules in the unit cell. This planar molecule features a C13-O2 double bond (1.2172(14) Å), with the angle C10-C13-O2 of 120.92(10)°. In the five-membered ring, the C1-N1 bond is 1.3061(15) Å, shorter than the C1-N2 bond 1.3819(15) Å.

III.2.3.5. Synthesis of 3-(*tert*-butyl)-5-(4-(1-methoxyethyl)phenyl)-1,2,4-oxadiazole (9)

3-(*tert*-Butyl)-5-(4-(1-methoxyethyl)phenyl)-1,2,4-oxadiazole (**9**) was obtained in very good yield starting from 4-(1-methoxyethyl)benzoic acid using the same reaction sequence as described in the general (Scheme 14).

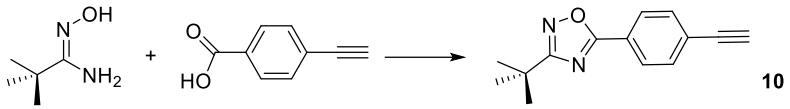


Scheme 14. Synthesis of 3-(*tert*-butyl)-5-(4-(1-methoxyethyl)phenyl)-1,2,4-oxadiazole (**9**).

The spectral data are also in agreement with the proposed molecular structure. The m/z shows the molecular peak at $M^+ = 260.2$ and some specific fragments e.g. M-CH_3 and M-(*t*-Bu). The ^{1}H NMR spectrum of **9** recorded in $CDCl_3$ features the specific aromatic signals along with a multiplet at 4.29 ppm attributed to the CH, two singlets (3.17 ppm, 1.38 ppm) for the methyl groups and the specific *t*-Bu singlet at 1.35 ppm.

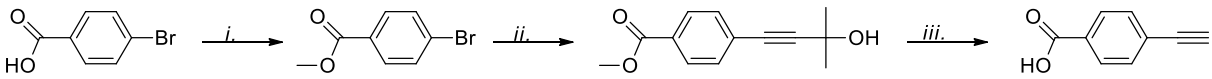
III.2.4. One step synthesis of 1,2,4-oxadiazoles bearing unsaturated rests

III.2.4.1. Synthesis of 3-(tert-butyl)-5-(4-ethynylphenyl)-1,2,4-oxadiazole (10)



Scheme 15. Synthesis of 3-(*tert*-butyl)-5-(4-ethynylphenyl)-1,2,4-oxadiazole (**10**).

Because of the fact that 4-ethynylbenzoic acid was used on a multi-gram scale and it is expensive, it was therefore necessary to synthetize it.[134] A modified synthetic route was developed that is much cheaper and more convenient than previously described methods and furnishes essentially quantitative yields. I took advantage of the sensitivity of the ester linkage toward potassium or sodium hydroxide to cleave the 2-hydroxypropyl group and saponify the ester simultaneously in l-butanol or 2-propanol to prepare 4-ethynylbenzoic acid in high yield. Methyl *p*-bromobenzoate (MBB) was mixed with a small excess of 2-methyl-3-butyn-2-ol in a deoxygenated, dried triethylamine/pyridine mixture (volume ratio 5/2) in the presence of catalytic amounts of dichloro-bis(triphenylphosphine)palladium, triphenylphosphine, and cuprous iodide, and the solution was refluxed; the sodium or potassium salt of ethynylbenzoic acid precipitated quantitatively from the reaction mixture. Crystalline ethynylbenzoic acid changes color upon standing from colorless to off-white and then to light tan, which suggested that it is not very stable at room temperature and light (it polymerizes slowly). In contrast its sodium and potassium salts are stable.



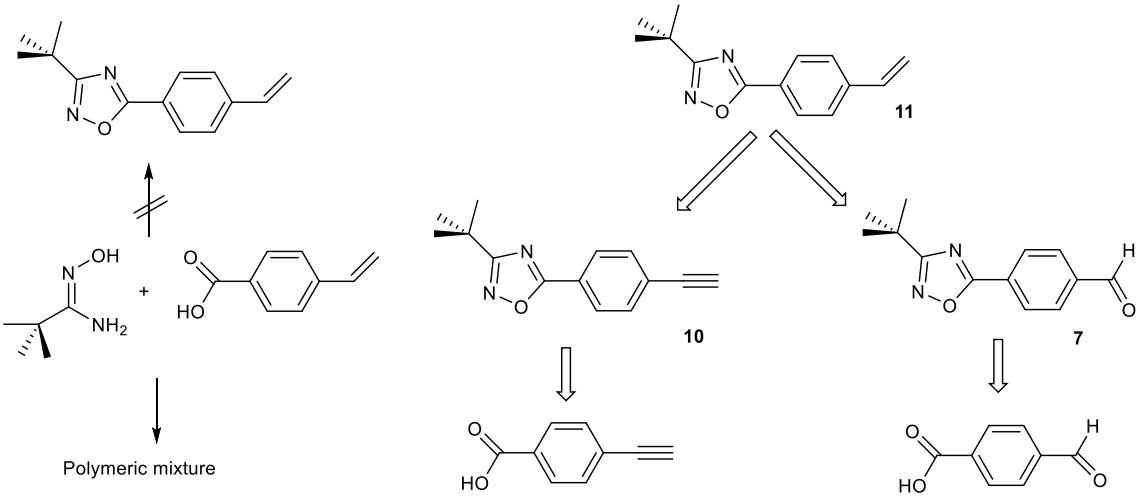
Scheme 16. Synthetic route for 4-ethynylbenzoic acid; *i*. MeOH, H_2SO_4; *ii*. $HOC(CH_3)_2CCH$, $PdCl_2(PPh_3)_2$, Et_3N; *iii*. NaOH, BuOH, HCl.

The ^1H NMR spectrum of **10** recorded in $CDCl_3$ reveals two typical 1,4-substituted aromatic signals and two singlets, one at 3.26 ppm correlated to the alkyne proton and the other one at 1.43 ppm corresponding to *t*-Bu group. Also the ^{13}C NMR spectrum shows clearly the typical alkyne signals at 82.69 ppm (Cq) and 80.25 ppm (CH).

III.2.4.2. Synthesis of 3-*tert*-butyl-5-(4-vinylphenyl)-1,2,4-oxadiazole (11)

The general procedure is reliable for most of the benzoic acid derivatives, but in some cases the nature of the substituents on the phenolic ring renders synthetic transformations more challenging. In some cases the substituent results in side reactions that disturb the cyclisation step or dramatically diminish the isolated yields. In other cases the benzoic acid derivative is either not commercially available or it is too expensive. Therefore starting from an already in 4-position substituted produced 1,2,4-oxadiazoles, new molecules were designed, isolated and characterized.

The first example is the 3-*tert*-butyl-5-(4-vinylphenyl)-1,2,4-oxadiazole (**11**) that cannot be obtained from the corresponding 4-vinylbenzoic acid using the general synthetic route outlined above. In this case the cyclisation takes place, but a polymeric mixture of the 1,2,4-oxadiazole compound is formed (Scheme 17).



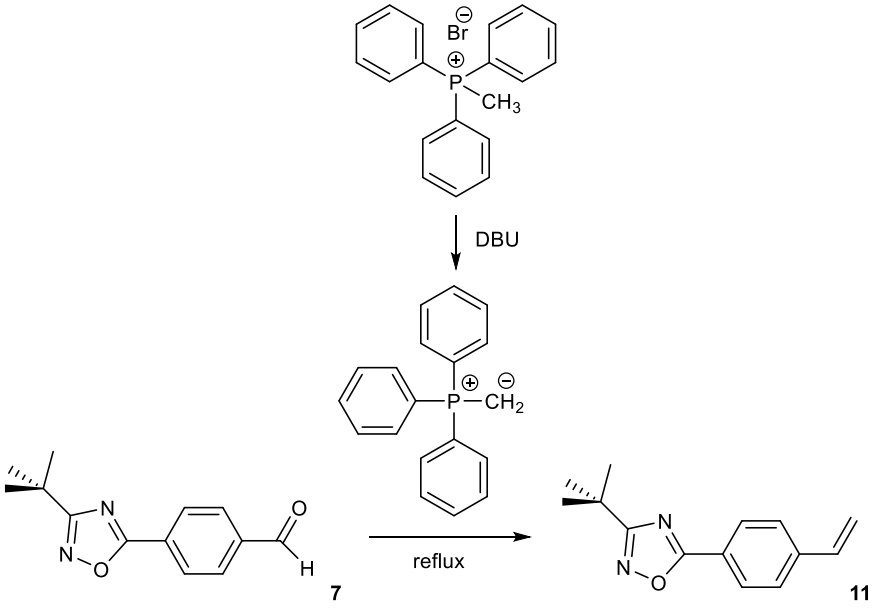
Scheme 17. Synthetic routes for the synthesis of and 3-*tert*-butyl-5-(4-vinylphenyl)-1,2,4-oxadiazole (**11**).

For this reason I had to develop other methods to prepare compound **11**. Two alternative, synthetic routes using as intermediates 3-*tert*-butyl-5-(4-ethynylphenyl)-1,2,4-oxadiazole (**10**) and 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)benzaldehyde (**7**) are shown in scheme 17. For one of these routes the selective hydrogenation of (**10**) using Lindlar catalyst was considered, but the disadvantage of this method was that in the final step a mixture of **11** and 3-*tert*-butyl-5-(4-ethylphenyl)-1,2,4-oxadiazole (**12**) was obtained. For the second synthetic path, the 4-formylbenzoic acid used as precursor is commercially available, but the yields for the transformation are low. Both methods were tested in parallel.

After isolation of 3-*tert*-butyl-5-(4-ethynylphenyl)-1,2,4-oxadiazole (**10**) the next step was the hydrogenation in the presence of the Lindlar catalyst. Performing the reaction at low

temperature (-10°C) and with a short reaction time (30 min) in THF as solvent the desired vinyl oxadiazole could be obtained as the major product.

The second route to afford 3-*tert*-butyl-5-(4-vinylphenyl)-1,2,4-oxadiazole (**11**) started from the 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)-benzaldehyde (**7**), which was converted a Witting reaction.[135]



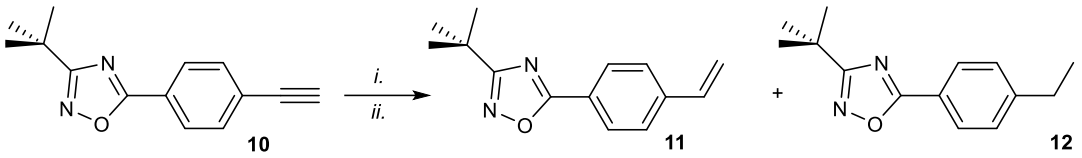
Scheme 18. Synthesis of 3-*tert*-butyl-5-(4-vinylphenyl)-1,2,4-oxadiazole (**11**) starting from **7**.

The reaction of methyltriphenyl-phosphonium bromide with DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) as a base in refluxing tetrahydrofuran or toluene afforded methylenetriphenylphosphorane, which further reacted with benzaldehydes to give the styrene derivative in good yield. Several amine bases (triethylamine, pyridine, DBN and DBU) were screened for the synthesis of 3-*tert*-butyl-5-(4-vinylphenyl)-1,2,4-oxadiazole (**11**) starting from with methyltriphenylphosphonium bromide and 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)-benzaldehyde (**7**). However, only DBU provided the product **11** in high yield and purity.

III.2.4.3. Synthesis of 3-*tert*-butyl-5-(4-ethylphenyl)-1,2,4-oxadiazole (12)

The hydrogenation process of 3-*tert*-butyl-5-(4-ethynylphenyl)-1,2,4-oxadiazole in the presence of the Lindlar catalyst is unfortunately not very selective. The vinyl group is also hydrogenated to an ethyl group. Higher temperatures and prolonged reaction time only result in increasing quantities of the ethyl derivate. After 2h at room temperature complete hydrogenation to the 3-*tert*-butyl-5-(4-ethylphenyl)-1,2,4-oxadiazole (**12**) was achieved and every attempt to avoid the formation of **12** failed. Furthermore separation of **12** from the vinyl

derivative **11** cannot be achieved because of very similar polarities of these compounds. After 0.5 h at -10°C the starting alkyne was still present in the reaction mixture (Scheme 19).

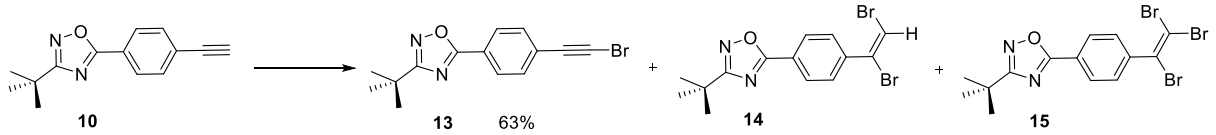


Scheme 19. Hydrogenation of 3-*tert*-butyl-5-(4-ethynylphenyl)-1,2,4-oxadiazole. *i*: H_2, Lindlar catalyst, THF, -10º C, 30 min, 90% (**11**) / 10% (**11**); *ii*: H_2, Lindlar catalyst, THF, r.t., 100 % (**12**).

III.2.4.4. Synthesis and structural characterization of alkynyl bromides derivates

III.2.4.4.1. Synthesis of 3-tert-butyl-5-(4-ethylphenyl)-1,2,4-oxadiazole (13)

As shown in Scheme 20, 5-(4-(bromoethynyl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (**13**) was synthesized from the 3-(*tert*-butyl)-5-(4-ethynylphenyl)-1,2,4-oxadiazole (**10**) at low temperature (0°C) and *N*-bromosuccinimide (NBS) as bromine source.



Scheme 20. Synthesis of 5-(4-(bromoethynyl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (**13**) starting from **10**.

Even if the mono-bromide derivative is preferentially formed, the formation of the more brominated derivatives (**14** and **15**) was unavoidable. The best yield obtained for the mono brominated compound was 63%. In the ^{1}H NMR spectrum of **13** recorded in $CDCl_3$, the signal at 3.26 ppm which belongs to the alkyne proton is absent and the masse m/z = 304/306 clearly shows the characteristic isotopic distribution due to the presence of bromine. The mono-brominated by-product (*E*)-3-(*tert*-butyl)-5-(4-(1,2-dibromovinyl)phenyl)-1,2,4-oxadiazole (**14**) is detected in the ^{1}H NMR spectrum at 7.23 ppm corresponding to the vinylic CH-proton. Mass spectrometry clearly shows the presence of two brome atoms in the molecule (m/z = 384/386). The 3-(*tert*-butyl)-5-(4-(1,2,2-tribromovinyl)phenyl)-1,2,4-oxadiazole (**15**) give rise to a simple ^{1}H NMR spectrum, four aromatic and nine *t*-butyl protons and the mass of m/z = 464/466. Crystals suitable for X-ray diffraction analysis for compound **13** were obtained by slow diffusion of hexane into a concentrated solution of **13** in DCM.

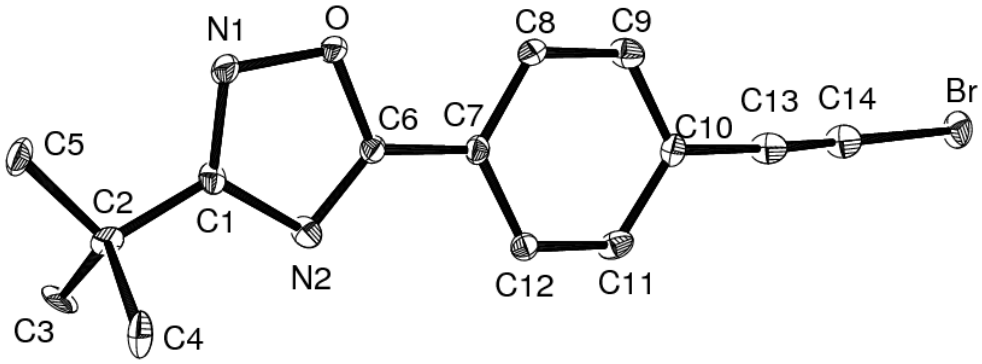


Figure 33. Molecular structure of 5-(4-(bromoethynyl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (**13**). Atoms are drawn as 50% thermal ellipsoids. Selected bond lengths [Å] and angles [°]: C1-N1 1.3031(16), C1-N2 1.3939(16), N1-O 1.4150(12), C6-O 1.3438(14), C6-N2 1.3030(15), C14-Br 1.8099(13), C13-C14-Br 177.1(6).

The solid-state molecular structure of **13** was established by X-ray diffraction analysis and shown in Figure 33. The compound crystallizes in the orthorhombic space group *P*na2$_1$. The planar molecule features a C14-Br bond length of 1.8099(13) Å and a C13-C14-Br angle of 177.1(6)°.

III.2.5. Synthesis of second generation 1,2,4-oxadiazoles

III.2.5.1. Synthesis of 1,2,4-oxadiazole derivatives bearing amidine and tioamide functional groups

III.2.5.1.1. Background

In pharmaceutical chemistry, amidoximes, amidine and thioamides are important precursors for the synthesis of several heterocycles, such as oxadiazole, imidazole and thiazole, all of which are known to be bioactive scaffolds.

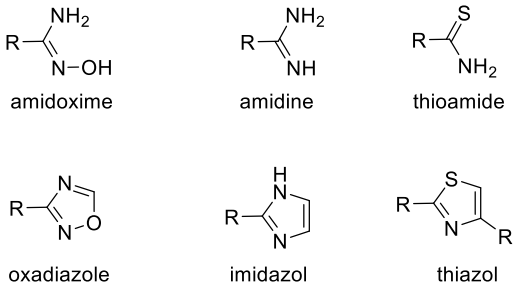


Figure 34. Structural representation of amidoxime, amidine, thioamide and the correspondent heterocycles.

In addition, thioamides derivatives show antitumor activity,[136] for example 6-mercaptopurine, marketed as Purinethol, is used in the treatment of lymphocytic leukemia.

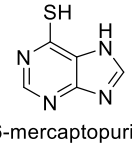
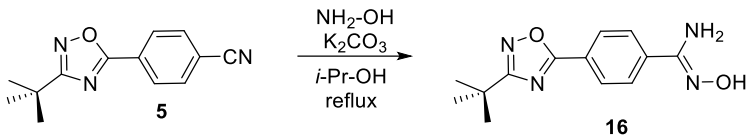


Figure 35. Structural representation of 6-mercaptopurine.

III.2.5.1.2. Synthesis of (Z)-4-(3-(tert-butyl)-1,2,4-oxadiazol-5-yl)-N' hydroxy-benzimid-amide (16)

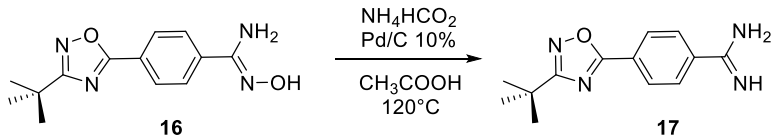
Many synthetic routes have been reported in the literature for the synthesis of amidoximes,[137-140] but one of the more common methods represents the addition of hydroxylamine to a nitrile (Scheme 21).



Scheme 21. Synthesis of (*Z*)-4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)-N'-hydroxybenzimidamide (**16**).

The amidoxime (*Z*)-4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)-N'-hydroxybenzimidamide (**16**) was prepared by treatment of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzonitrile with hydroxylamine in the presence of K_2CO_3 in refluxing *iso*-propanol. Although other bases (K_3PO_4, NaOMe, NaOH) and solvents (ethanol, methanol) were used, the best yields were obtained with isopropyl alcohol as the appropriate solvent, which ensured homogeneity of the reaction mixture during the reaction (64% yield). The compound shows in the ^{1}H NMR spectrum four protons in the aromatic region, two broad signals at 4.9 ppm and 1.62 ppm integrating for two and one protons of the amino and hydroxyl groups, respectively, and the 1.44 ppm signal attributed to the nine protons of the *t*-butyl group. The MS shows m/z = 260.1 (M^+).III.2.5.1.3. Synthesis of (*Z*)-4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)-N' hydroxy-benzimid-amide (17)

Amidine derivatives are usually prepared from nitriles using one of the several reported pathways, such as the Pinner method,[141] transformation via a thioimidate [142] or by alkylchloroaluminium amides reaction.

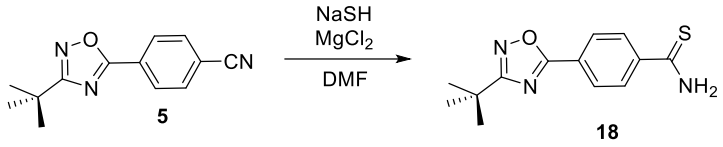


Scheme 22. Synthesis of (*Z*)-4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)-N' hydroxy-benzimidamide (**17**).

More elegantly, Anbazhagan *et al.* generated amidine compounds by reduction of amidoximes.[143] They have explored several hydrogen transfer reagent such as ammonium formate which circumvents hydrogen gas for the conversion of amidoximes to amidines. Definitely, ammonium formate in the presence of Pd/C system reduces amidoximes,

providing the corresponding amidines in good yield (69%). 4-(3-(*tert*-Butyl)-1,2,4-oxadiazol-5-yl)benzimidamide (**17**) was obtained starting from (*Z*)-4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)-*N*'-hydroxybenzimidamide (**16**) using the above described method. The ^{1}H NMR spectrum indicates four protons in the aromatic zone, one broad signal accounting for three protons of the amidine group at 4.95-5.45 ppm and the 1.44 ppm signal attributed to the nine protons of the *t*-butyl group. The MS-ESI spectrum gives a mass of 245.13 ($M+H^{+}$).

III.2.5.1.4. Synthesis of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzothioamide (18)



Scheme 23. Synthesis of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzothioamide (**18**).

The straightforward reaction of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzonitrile (**5**) with sodium hydrogen sulfide and magnesium chloride in dimethylformamide (DMF) generated the new 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzothioamide (**18**) in excellent yield (92%).[144]

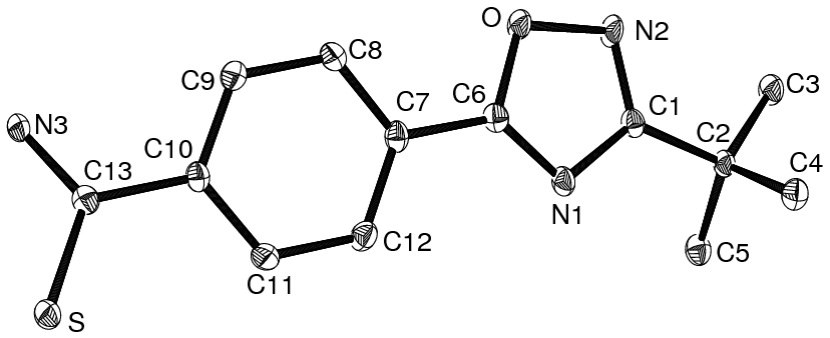


Figure 36. Molecular structure of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzothioamide (**18**). Atoms are drawn as 50% thermal ellipsoids. Selected bond lengths [Å] and angles [°]: C10-C13 1.496(2), N3-C13 1.322(19), C13-S 1.675(15), C10-C13-N3 115.53(13)°, N3-C13-S 122.92(12)°, C10-C13-S 121.54(11)°.

The solid-state molecular structure of **18** was established by X-ray diffraction analysis and shown in Figure 36. The compound **18** crystallizes in the triclinic space group *P*-1 with one molecule in the asymmetric unit. All bond distances and angles are within the expected range.

III.2.5.2. Synthesis of 1,2,4-oxadiazole derivatives bearing hydroxylamine moieties

III.2.5.2.1. Background

Various phenoxyamine derivatives proved to be biologically active and consequently employed as herbicides and antiproliferative agents.[145-148]

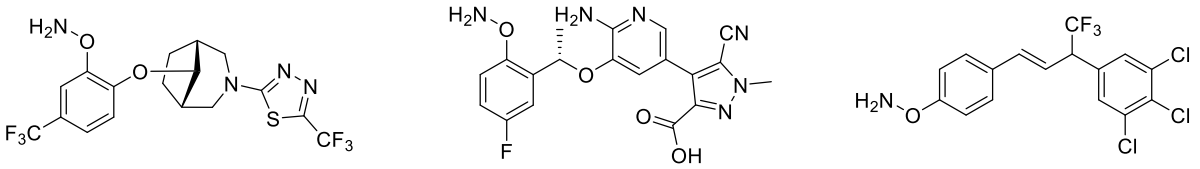
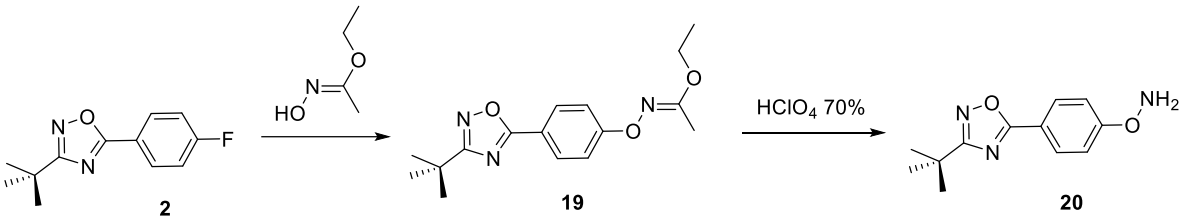


Figure 37: Examples of phenoxyamines which exhibit biological activity.

N-arylhydroxylamines are also considered important precursors and intermediates for several other research branches such as polymerization inhibitors,[149,150] reagents [151-155] and bioactive pharmaceuticals.[156,157]

III.2.5.2.2. Synthesis of *O*-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)hydroxylamine (20)

Phenoxyamines have been obtained by reacting the fluorobenzenes and ethyl acetohydroxamate (Zinner's method)[158,159] or from *t*-butyl-*N*-hydroxycarbamate (Carpino's method)[160] with a base followed by hydrolysis with an acid. A number of substituted phenoxyamine derivatives have been synthesized by these methods. However, this reaction is limited to phenoxyamine derivatives bearing strong electron-withdrawing substituents in *ortho*- and/or *para*-position such as 2- or 4-nitrophenoxy-, 2,4- or 2,6-dinitrophenoxy- and 2,4,6-trinitrophenoxy-amines.[161]



Scheme 24. Synthesis of *O*-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)hydroxylamine (**20**).

Since the method developed by Zinner is reproducible and ethylacetohydroxamate is commercially available, we investigated the synthesis of *O*-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)hydroxylamine (**20**) starting from 3-(*tert*-butyl)-5-(4-fluorophenyl)-1,2,4-oxadiazole (**2**). The first reaction step is the substitution of 3-(*tert*-butyl)-5-(4-fluorophenyl)-1,2,4-oxadiazole by the potassium salt of ethylacetohydroxamate in DMF. The intermediate

ethyl (*E*)-*N*-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenoxy)acetimidate (**19**) was isolated in a good yield (68%).

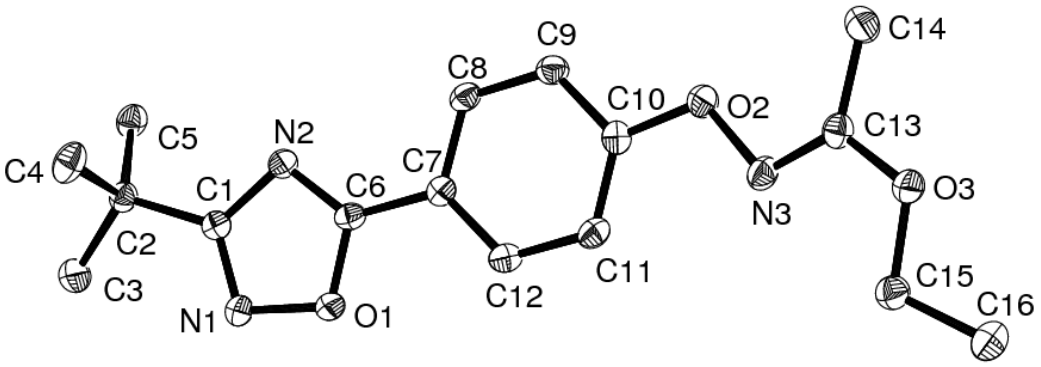


Figure 38. Molecular structure of ethyl (*E*)-N-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenoxy)acetimidate (**19**). Atoms are drawn as 50% thermal ellipsoids. Selected bond lengths [Å] and angles [°]: C10-O2 1.371(12), O2-N3 1.440(11), N3-C13 1.279(13), C13-C14 1.489(15), C10-O2-N3 111.69(7)°, O2-N3-C13 108.06(8)°, N3-C13-C14 113.18(9)°.

The solid-state molecular structure of **19** was established by X-ray diffraction analysis and is shown in Figure 38. The compound **19** crystallizes by slow evaporation of diethyl ether in the triclinic space group *P*-1 with one molecule in the asymmetric unit. The short value of the N3-C13 bond (1.279 Å) definitely is consistent with a double bond.

In the second step, the acetimidate derivative **19** was reacted with aqueous $HClO_4$ (70%) in dioxane at room temperature to afford *O*-(4-(3-(*ter*t-butyl)-1,2,4-oxadiazol-5-yl)phenyl)hydroxylamine (**20**) in moderate yield (51%). The ^1H NMR spectrum shows the disappearence of the acetamidate group (CH_3 as a singlet at 2.14 ppm and the CH_2-CH_3 from 4.25 ppm and 1.37 ppm) and the formation of a brought signal attributed to the NH_2 group at 6.11-5.82 ppm. The mass spectra also changed from 303.1 (M^+), mass of the intermediate, to 233.1 (M^+) the mass of the hydroxylamine derivative.

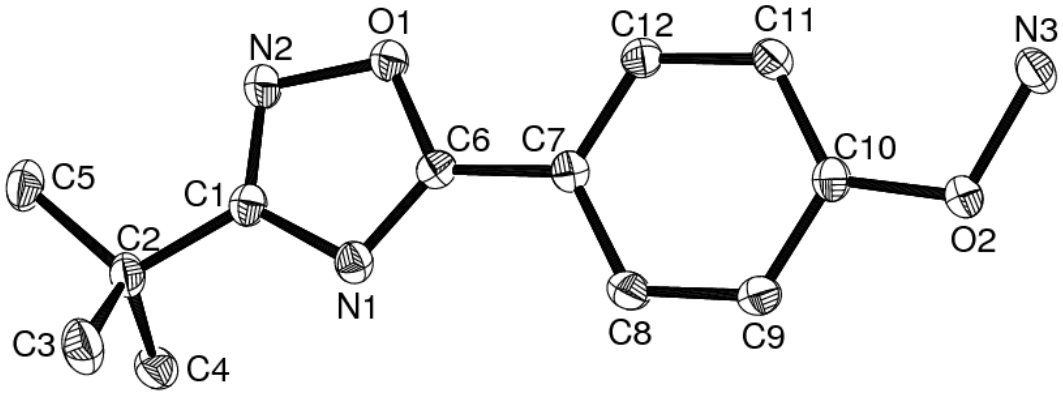
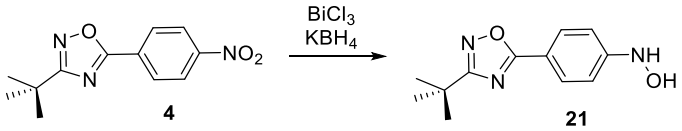


Figure 39. Molecular structure of ethyl *O*-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)hydroxylamine (**20**). Atoms are drawn as 50% thermal ellipsoids. Selected bond lengths [Å] and angles [°]: O2-C10 1.371(15), O2-N3 1.457(14), C10-O2-N3 113.09(10).

The solid-state structure of **20** was established by X-ray diffraction analysis and is depicted in Figure 39. The compound **20** crystallizes from a concentrated DCM solution at room temperature in the orthorhombic space group $P2_12_12_1$ with one molecule in the asymmetric unit. The molecule is almost planar with the length bonds and angles in the expected range.

III.2.5.2.3. Synthesis *N*-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)hydroxylamine (21)

A possible synthetic pathway of N-arylhydroxylamines starts from nitroarenes. This process can lead to nitrosoarenes, azoarenes, azoxyarenes, hydroxylamines or amines. In order to selectively reduce this unit several reducing agents were screened.[162] One of the most successful reagents represents the mixture between sodium borohydride and metal salts, such as $NaBH_4/CoCl_2$,[163] $NaBH_4/Cu(OAc)_2$,[164] $NaBH_4/Sb$,[165] $NaBH_4/NiCI_2$,[166] $NaBH_4/BiCl_3$.[167]



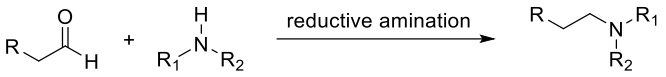
Scheme 25. Synthesis of *N*-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)hydroxylamine (**21**).

In order to generate *N*-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)hydroxylamine (**21**) we treated the already prepared 3-(*tert*-butyl)-5-(4-nitrophenyl)-1,2,4-oxadiazole (**4**) with the $BiCl_3/KBH_4$ mixture at room temperature in ethanol/water (Scheme 25). The hydroxylamine derivative was isolated in good yields (76%) and the spectral data confirmed the proposed molecular structure. The mass recorded is 233.1 (M^+) and the ^1H NMR spectrum shows the OH proton at 6.49 ppm. The NH proton was detected along with other two aromatic protons at 7.14-6.96 ppm.

III.2.5.3. Synthesis of 1,2,4-oxadiazole derivatives bearing secondary amine units

III.2.5.3.1. Background

The synthesis of new compounds containing secondary amines functionalities, important intermediates in pharmaceutical and fine chemicals development [168] is an attractive area in modern organic and medicinal chemistry synthesis. The reductive amination, in which a carbonyl unit is transformed into a tertiary amine, represents one of the most frequently used synthetic routes for the production of amines in the pharmaceutical industry.



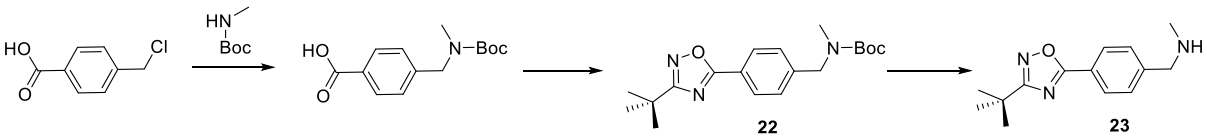
Scheme 26. The reductive amination of an aldehyde with a secondary amine.

Having this motivation in mind, it was planed the synthesis of two secondary amines derivatives from 1,2,4-oxadiazole, a long chained (1-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-*N*-methylmethanamine) and a short chain derivative (4-(3-(tert-butyl)-1,2,4-oxadiazol-5-yl)-N-methylaniline) as illustrated in Figure 40.

Figure 40. 1,2,4-Oxadiazole targets functionalized with secondary amine.

III.2.5.3.2. Synthesis of 1-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-*N*-methylmethanamine (23)

The starting sequence for this compound involves also the preparation of 4-(((*tert*-butoxycarbonyl)(methyl)amino)methyl)benzoic acid, an exotic and quite expensive chemical compound. Starting from 4-(chloromethyl)benzoic acid and *tert*-butyl methylcarbamate, the wanted precursor was obtained without any problems.[169]

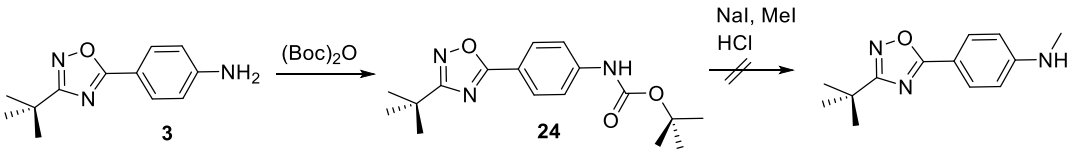


Scheme 27. Synthetic path of 1-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-*N*-methylmethanamine (**23**).

With the benzoic acid precursor in hand, 1-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-*N*-methylmethanamine (**23**) was synthesed in two steps. The first step was the cyclisation between the acid and the amidoxime in order to build the oxadiazole ring. The reaction worked well and at the end the *tert*-butyl (4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzyl)(methyl)carbamate (**22**) was isolated in an 86% yield. The second step was the classical elimination of the Boc group in the presence of HCl. The MS-EI shows m/z = 245.2 (M^+). The ^{1}H NMR reveals the presence of the aromatic protons, the -CH_2 at 3.84 ppm, the -CH_3 at 2.47 ppm and just one *t*-butyl group at 1.43 ppm (verifying that the Boc group was indeed cleaved).

III.2.5.3.3. Attempt for synthesis of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)-N-methylaniline

The reaction sequence is presented in Scheme 28. 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)aniline (**3**) was reacted with Boc-anhydride in basic conditions (NaOH) in order to deprotonate the molecule and to activate the anhydride. *tert*-Butyl-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)carbamate (**24**) was obtained in very good yield and purity.



Scheme 28. Synthetic path to 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)-N-methylaniline.

The second step was the introduction of the methyl function followed by the elimination of the Boc group under acid conditions, but somehow the introduction of the methyl group failed. The deprotonation with NaH and addition of MeI resulted in a dark solution from which the desired compound could be isolated. The reaction was repeated twice with the same outcomet. However, the intermediate *tert*-butyl-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)carbamate (**24**) was characterized and tested for antitumor activity for comparison with 1-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-*N*-methylmethanamine (**23**).

The MS-ESI shows 318.2 ($M + H^+$), 340.2 ($M + Na^+$) and 657.4 ($2xM + Na^+$). The ^1H NMR spectrum shows the disappearance of one proton from the $–NH_2$ group (the signal is shifted and the integration now accounts for one proton) and another singlet appears at 1.41 ppm for the Boc group.

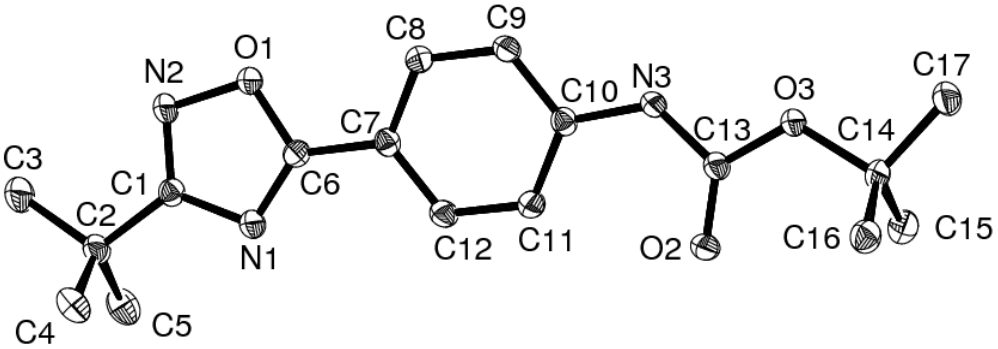


Figure 41. Molecular structure of *tert*-butyl-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)carbamate (**24**). Atoms are drawn as 50% thermal ellipsoids.

The solid-state structure of **24** was established by X-ray diffraction analysis and shown in Figure 41. The compound **24** crystallizes from a saturated chloroform solution in the

monoclinic space group $P2_1/c$ with one molecule in the asymmetric unit. The molecule presents the *tert*-butyl group out of the plan of the molecule. The length bonds and angles are unexceptional.

III.2.6. *In vitro* anti-tumor activity of second generation 1,2,4-oxadiazole

Compounds **13**, **17**, **18**, **20**, **21**, **23** and **24** were tested for *in vitro* anti-tumor activity against a panel of 12 cell lines was assessed using a monolayer cell survival and proliferation assay. As presented in Figure 42, the seven tested 1,2,4-oxadiazole compounds revealed not an impressive antitumoral activity against the panel of 12 cell lines. Clearly, the strongest activity from these seven candidates belongs to the alkynyl-bromide 1,2,4-oxadiazole derivative **13**. With a geometric mean IC_{50} value of 33.95 µM (Figure 42) this compound proved to be a moderate antitumor agent. The amidine **17** and thioamide **18** with mean IC_{50} value of 63.13 µM (**17**) and 57.16 µM (**18**), respectively, display a weak anti-tumor activity against the 12 cell lines tested. The remaining substances have only marginal or no anti-tumor activity.

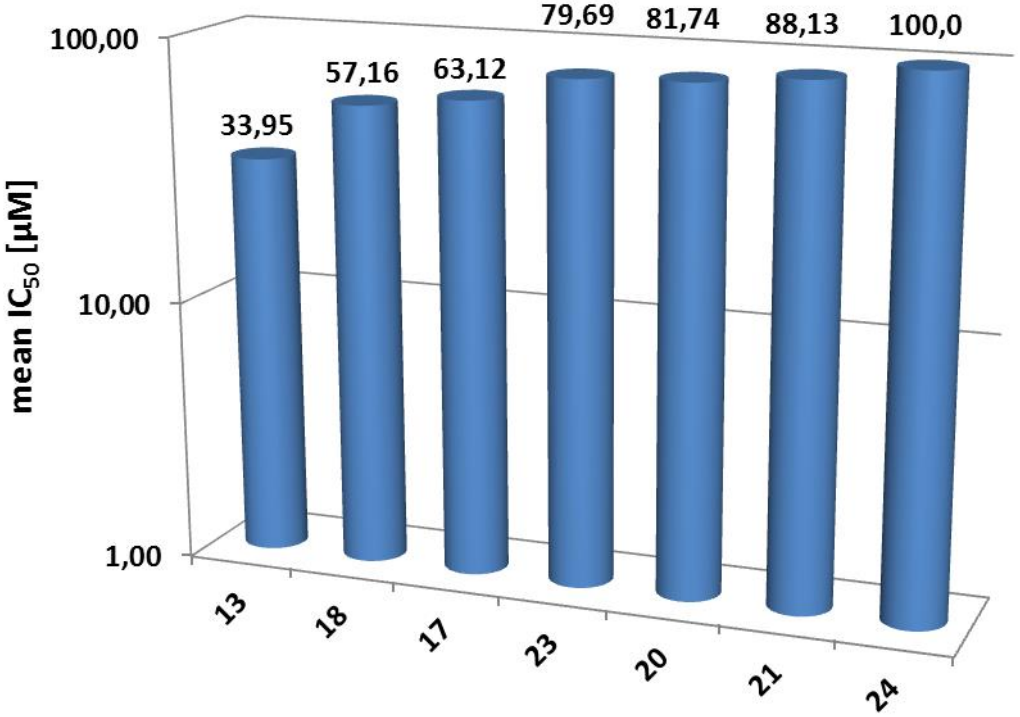


Figure 42. In vitro anti-tumor activity of compounds **13**, **17**, **18**, **20**, **21**, **23** and **24** towards 12 human tumor cell lines.

Figure 43 (Heatmap presentation of individual IC_{50} values) illustrates the tumor selectivity of the seven compounds investigated. It can be easily observed that **13**, although it has a moderate mean IC_{50}, it shows good selectivity against RXF 486 (renal tumor cell) and

MAXF 401 (mammary tumor cell) lines. Another compound that reveals moderate selectivity against the PRXF 22Rv1 (prostate tumor cell) line is the amidine **17**. Unfortunately, the remaining molecules exhibit no tumor selectivity.

compound	unit	Cell lines												Geom. mean IC_{50} [µM]
		CXF HT-29	GXF 251	LXFA 629	LXFL 529	MAXF 401	MEXF 462	OVXF 899	PAXF 1657	PRXF 22Rv1	PXF 1752	RXF 486	UXF 1138	
13	µM	92,77	32,19	41,80	58,12	9,74	20,87	100,0	31,78	37,00	64,88	6,14	34.04	**33,95**
18	µM	55,25	57,59	82,17	43,78	31,34	77,51	83,66	45,10	46,91	40,51	75,59	80,68	**57,16**
17	µM	80,55	43,81	89,92	46,44	82,00	90,18	100,0	78,01	28,82	42,32	100,0	38,61	**63,12**
23	µM	100,0	65,94	100,0	70,61	62,55	100,0	100,0	57,97	69,36	56,03	100,0	100,0	**79,69**
20	µM	100,0	44,37	98,29	53,77	86,88	86,79	100,0	89,12	71,76	78,69	100,0	100,0	**81,74**
21	µM	100,0	100,0	100,0	53,15	57,00	87,99	100,0	82,40	100,0	100,0	100,0	100,0	**88,13**
24	µM	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	**100,00**

1/32 1/16 1/8 1/4 1/2 1 2 4 8 16 32 -fold mean IC_{50}

sensitive cell lines — resistant cell lines

Figure 43. Heatmap presentation of individual IC_{50} values for compounds **13**, **17**, **18**, **20**, **21**, **23** and **24** in a panel of 12 human tumor cell lines.

III.3. 1,2,4-Oxadiazole compounds bearing cyclic and heterocyclic substituents

III.3.1. Synthesis of 1,2,4-oxadiazole derivatives bearing N-phenyl-maleimide or N-phenyl-succinimide functionalities

III.3.1.1. Background

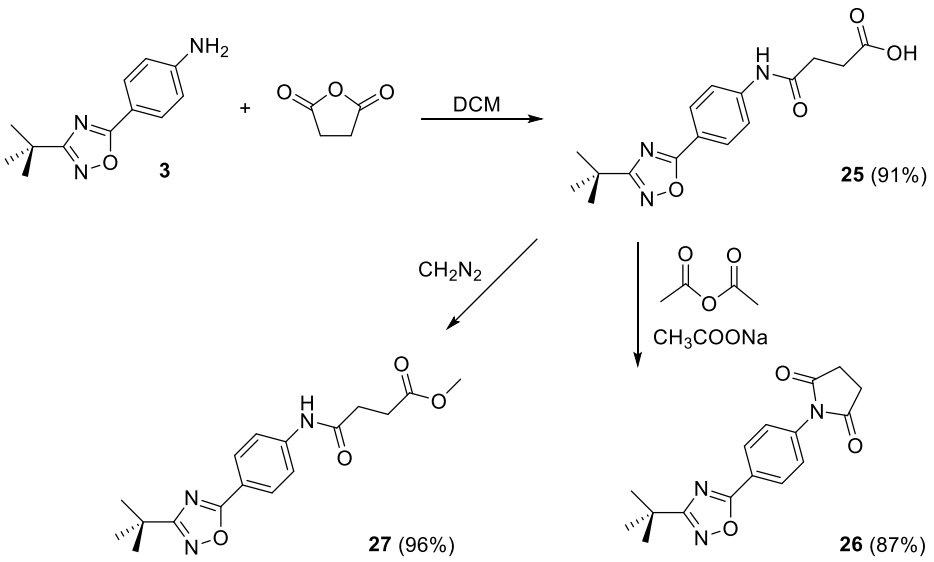
The maleimide motif is a five-menbered heterocycle with various applications in pharmacological chemistry. Kratz *et al*. synthesized maleimide derivatives of Doxorubicin and Camptothecin. After intravenous administration these designed anticancer drugs bind rapidly to circulating albumin.[170-172] Endogenous albumin could be seen as a drug carrier and according to the pathophysiology of tumor tissue it accumulates in solid tumors.[173,174] Therefore, designed prodrugs have a higher antitumor efficacy *in vivo* than the free drugs. Furthermore, maleimides possess strong antifungal activities against important human opportunistic pathogenic fungi. These antifungal drugs appear to be excellent candidates for further development.[175,176] Barrett *et al.* pointed out that the possibility of performing chemical modifications is a requirement to develop novel drugs and a strong activity is just the starting point.[177]

Another moiety worth investigating is succinimide, because *N*-phenylsuccinimides are regarded as one of the most efficacious agricultural fungicides.[178,179] They have also been shown to be selective nephrotoxic compounds.[180]

Taking into consideration the biological activity of natural products containing the 1,2,4-oxadiazol moiety, such as phidianidines A and B (selective inhibitors of DAT), it was worthy synthesizing, isolating and characterizing novel natural product analogues of 1,2,4-oxadiazole derivatives that carry *N*-phenyl-maleimide or *N*-phenyl-succinimide functionalities in order to improve the biological activity. The new derivatives have been tested for their *in vitro* anti-tumor activity towards a panel of 11 cell lines.

III.3.1.2. Synthesis of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)pyrrolidine-2,5-dione (26)

A variety of methods have been reported for the preparation of this class of compound. Their preparation essemtially follows a two-step protocol. First it is necessary to synthesize the amide derivative **25**. This step was performed under inert conditions by mixing an equimolar amount of aniline **3** and succinic anhydride in a minimum volume of dichloromethane (Scheme 29). Compound **25** was obtained after a short reaction time and in high yield (91%). The methy ester derivative, 4-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylamino)-4 oxobutanoate (**27**), was prepared by addition of a diethyl ether solution of diazomethane to a suspension of amide **25.** For the synthesis of imide **26** the starting material, amide **25**, was mixed with an equimolar amount of sodium acetate in acetic anhydride and the mixture was heated for 4 h at 80-85ºC; resulting in the corresponding *N*-aryl-succinimide **13** in very good yield (87%).



Scheme 29. Synthesis of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)pyrrolidine-2,5-dione (**26**).

The structures of compounds **26** and **27** were also confirmed by X-ray structure analysis (Figs. 44-45). In compound **26** the oxazole and phenyl rings are approximately

coplanar (6(12)°), but the pyrrolidine ring is rotated by 52(14)° with respect to the phenyl ring.

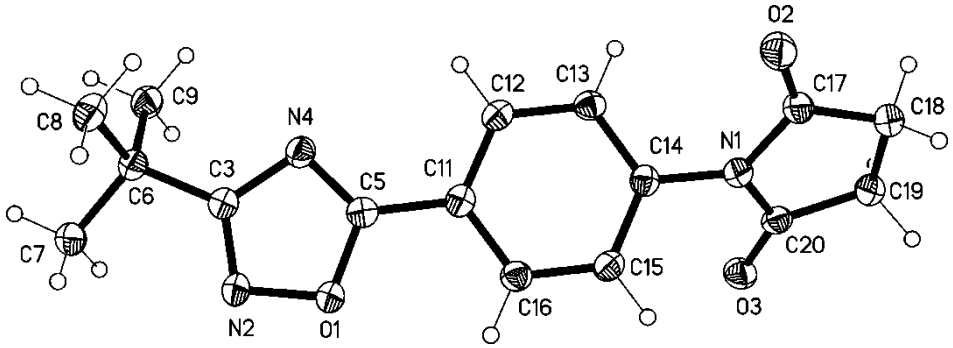


Figure 44. Molecular structure of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)pyrrolidine-2,5-dione (**26**). Atoms are drawn as 50% thermal ellipsoids.

The main packing interaction is an offset stacking parallel to the *c* axis (not shown). Compound **27** has an interplanar angle of 14(3)°; the molecules are associated into ribbons parallel to [110] by one long N–HΛN and two shorter C–HΛO interactions.

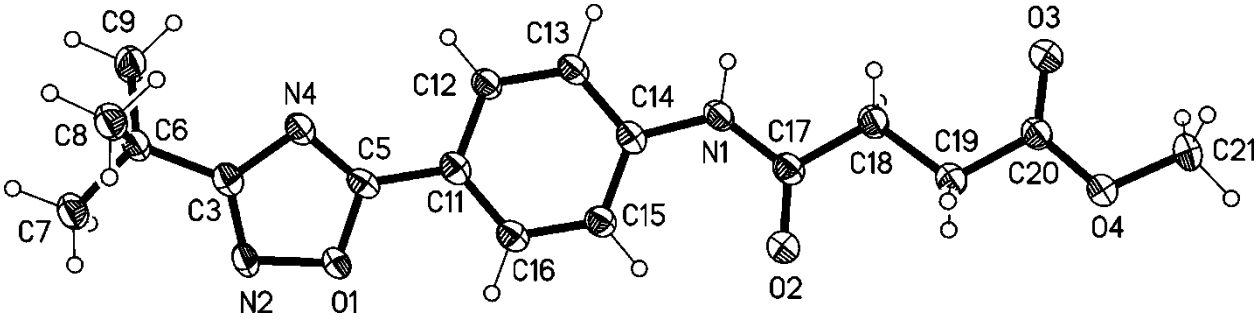


Figure 45. Molecular structure of 4-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylamino)-4 oxobutanoate (**27**). Atoms are drawn as 50% thermal ellipsoids.

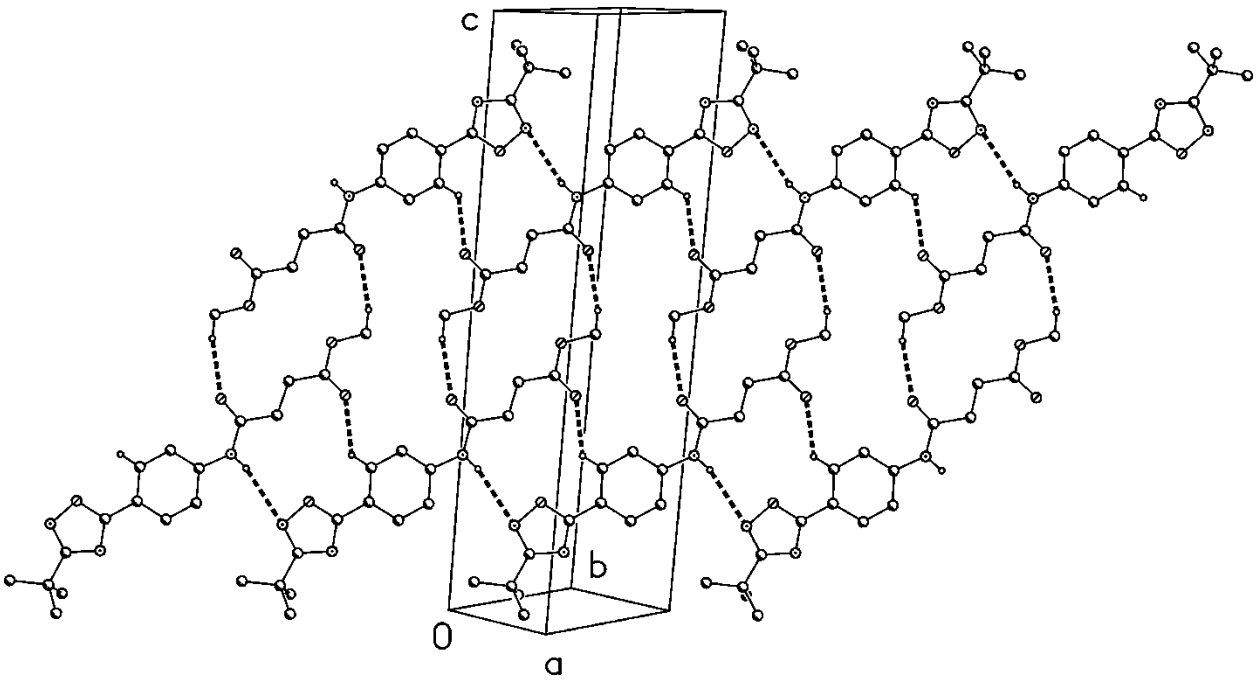
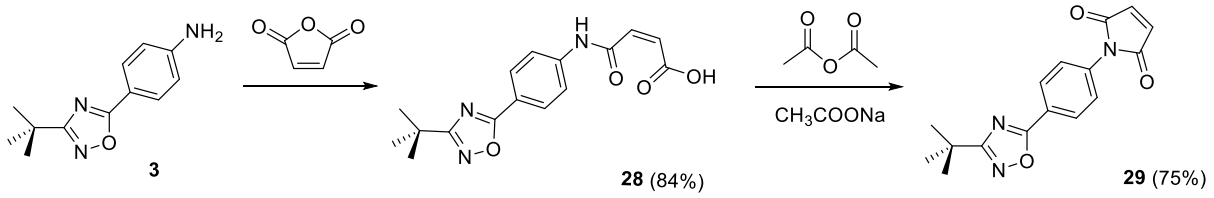


Figure 45a. Packing diagram of compound (**27**). Dashed lines indicate hydrogen bonds.

III.3.1.3. Synthesis of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-1H-pyrrole-2,5-dione (29)

The synthesis of amide **28** was performed by mixing an equimolar amount of aniline **8** and maleic anhydride in dichloromethane (Scheme 30). The amide **28** was obtained with a good yield (84%). For the synthesis of imide **29** the amide **28** was mixed with an equimolar amount of sodium acetate in acetic anhydride and the mixture was heated for 4 h at 80-85ºC.



Scheme 30. Synthesis of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-1*H*-pyrrole-2,5-dione (**29**).

The structure of compound **28** was also confirmed by X-ray structure analysis (Figs. 46, 46a). In compound **28** the interplanar angle is 11°; the intramolecular hydrogen bond is almost symmetrical (O4 – H04 1.03(3), H04ΛO2 1.47(3) Å). The molecules are linked to form layers perpendicular to the hexagonal *c* axis by one C–HΛO and one three-centre (N–H, C–H) ΛO interaction. The *Z*-configuration of the double bond is unequivocally.

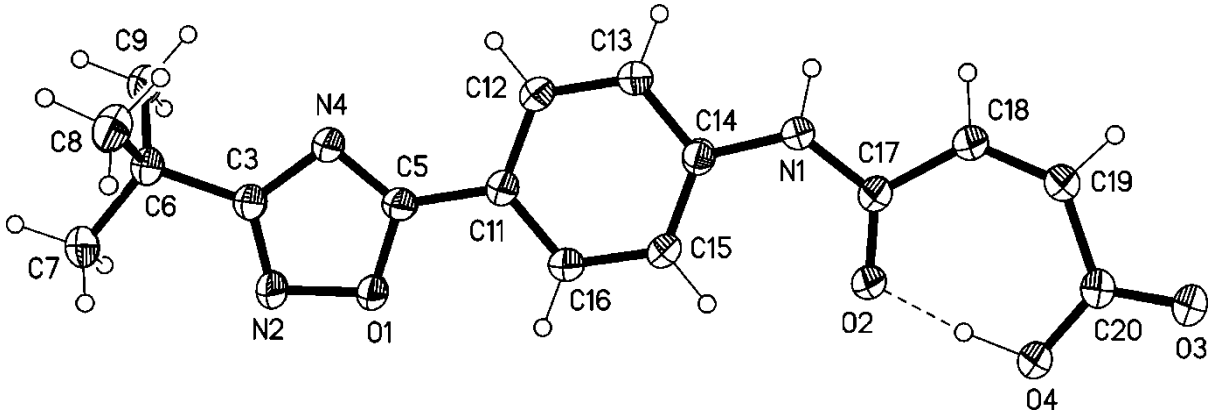


Figure 46. Molecular structure of (*Z*)-4-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylamino)-4-oxobut-2-enoic acid (**28**). Atoms are drawn as 50% thermal ellipsoids.

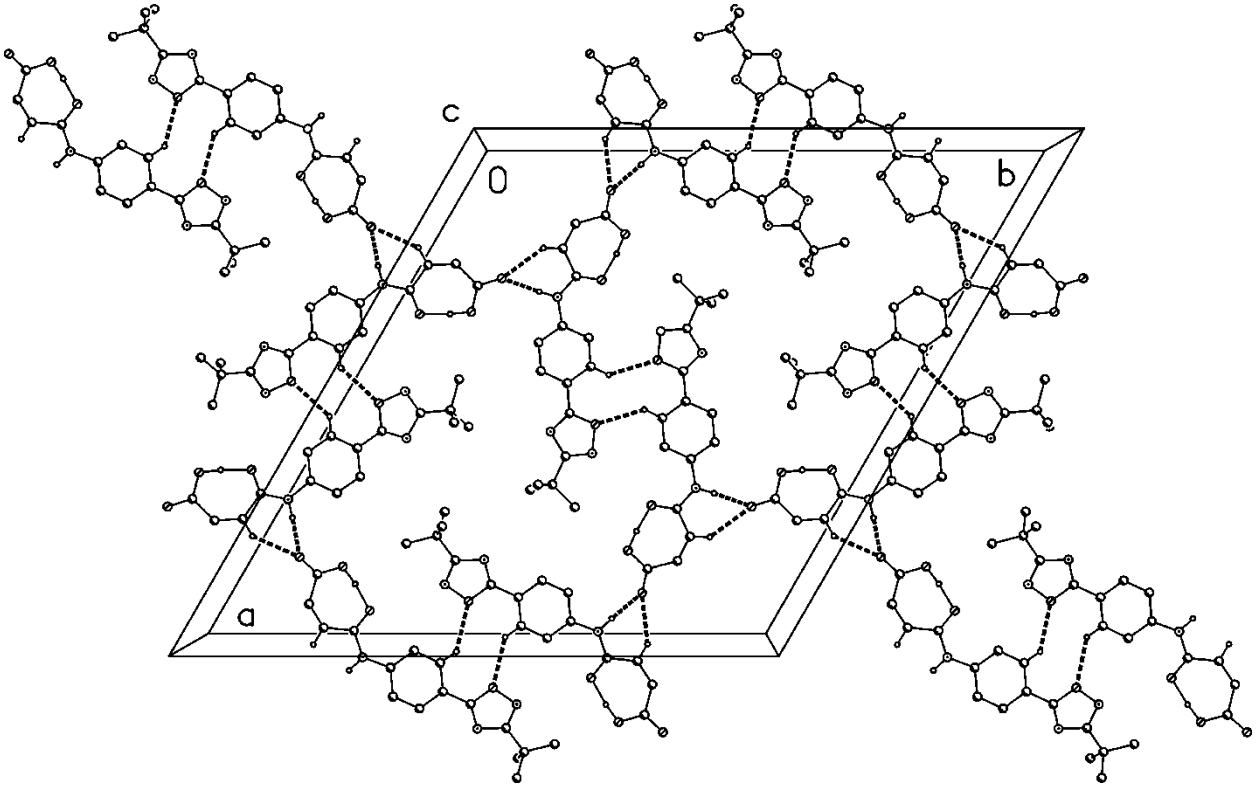


Figure 46a. Packing diagram of compound **28**. Dashed lines indicate hydrogen bonds.

III.3.1.4. *In vitro* anti-tumor activity towards human tumor cell lines of *N*-phenyl-maleimide or *N*-phenyl-succinimide derivatives and intermediates.

In vitro anti-tumor activity of synthesized compounds **3**, **25-29** towards a panel of 11 cell lines was assessed using a monolayer cell survival and proliferation assay. Derivative **29** was with a mean IC_{50} value of 9.4 µM the most potent compound. In contrast compounds **3**, **27**, **26**, **28** and **25** exhibit only marginal anti-tumor activity towards the 11 cell lines investigated (Figure 47).

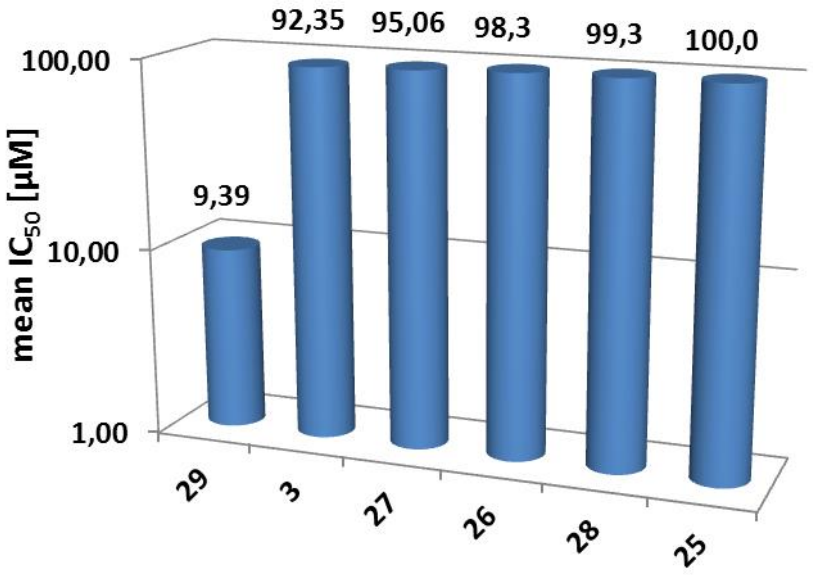


Figure 47. In vitro anti-tumor activity of compounds **3**, **25-29** towards 11 human tumor cell lines.

Activity screening of different compounds in a cell line panel with various tumor histo-types enable us to analyse their protency and tumor selectivity. Promissing candidates can then be taken to further be developed. The tumor selectivity of the compounds is illustrated in Figure 48, representing a heat-map presentation of the individual IC_{50} values. Overall, good antitumor potency (mean IC_{50} < 30µM) combined with good selectivity (range of activity > 8 or at least 2 above-average sensitive cell lines) was found for **29**.

compound	unit	Cell lines											Geom. mean IC_{50} [µM]
		CXF HT-29	GXF 251	LXFA 629	LXFL 529	MAXF 401	MEXF 462	OVXF 899	PAXF 1657	PXF 1752	RXF 486	UXF 1138	
29	µM	15,0	28,1	22,0	6,9	9,3	7,8	10,9	3,9	11,1	4,5	5,0	**9,4**
3	µM	100,0	55,3	100,0	76,8	98,1	100,0	100,0	100,0	100,0	100,0	100,0	**92,4**
27	µM	100,0	100,0	100,0	57,3	100,0	100,0	100,0	100,0	100,0	100,0	100,0	**95,1**
26	µM	100,0	100,0	100,0	82,9	100,0	100,0	100,0	100,0	100,0	100,0	100,0	**98,3**
28	µM	100,0	92,4	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	**99,3**
25	µM	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	**100,0**

1/32 1/16 1/8 1/4 1/2 1 2 4 8 16 32 -fold mean IC_{50}

sensitive cell lines — resistant cell lines

Figure 48. Individual IC_{50} values [µM] of compounds **3**, **25**-**29** in a panel of 11 human tumor cell lines.

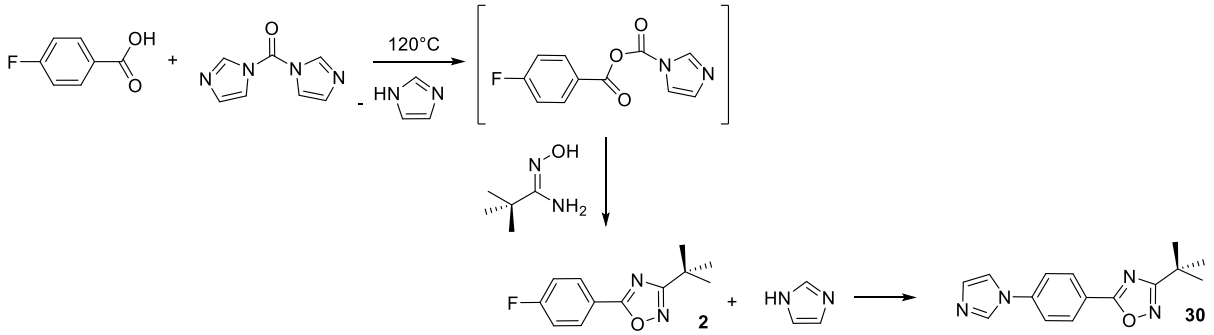
III.3.2. Synthesis of 1,2,4-oxadiazole derivatives bearing imidazole moiety

III.3.2.1. Background

The five-membered heterocyclic imidazole moiety can be found in a wide range of natural products.[181-183] Its bioactivity has made it a popular substructure in various synthetic products such as fungicides and herbicides,[184,185] plant growth regulators[186] and curative drug compounds.[187,188] In green chemistry and organometallic chemistry imidazoles have found applications as ionic liquids[189-192] and imidazole-related N-heterocyclic carbenes.[193-197] Although aromatic substituted imidazoles are important precursors for ionic liquids and N-heterocyclic carbenes, only a limited number of synthetic protocols are known.[198,199] Many of the employed protocols are either too expensive for large scale production or furnish only very low yields.

III.3.2.2. Synthesis of 5-(4-(1*H*-imidazol-1-yl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (30)

During the synthetisof 3-(*tert*-butyl)-5-(4-fluorophenyl)-1,2,4-oxadiazole (**2**) it was noted that a by-product precipitated during work-up and the side product was identified as 5-(4-(1*H*-imidazol-1-yl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (**30**).

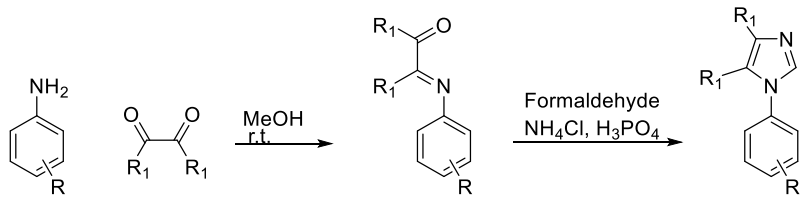


Scheme 31. Synthesis of 5-(4-(1*H*-imidazol-1-yl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole via the F-derivative **2**.

Its formation is rationalized in Scheme 31. Activation of the 4-fluorobenzoic with carbonyldiimidazole (CDI) generates imidazole which reacts with **2**to give 5-(4-(1*H*-imidazol-1-yl)phenyl)-3-(*ter*t-butyl)-1,2,4-oxadiazole (**30**). Unfortunately, this synthesis is not very reproducible with yields varying from 8 to 50%.

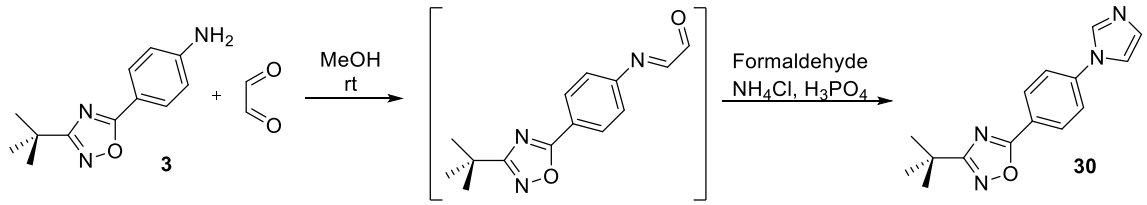
Grindev *et al.* prepared a series of imidazoles by a simple procedure in which aqueous solution of glyoxal, formaldehyde and ammonium salts of amines were heated to 95°C.[198] Following this method, it was possible to form the desired imidazoles with an aromatic substituent in 1-position relatively low yield (14%).

Gardiner modified the procedure by adding ammonium chloride and by using dioxane in the solvent mixture.[199] However, in my case similar yields as obtained by Gridnev's method were observed. Furthermore, increasing the reaction time and raising the temperature did also not improve the reaction outcome. Finally the conditions described by Liu *et al.* were explored.[200] This group developed a two-step protocol for the synthesis of sterically hindered imidazoles with acceptable yields.



Scheme 32. Preparation of 1-arylimidazoles according to Liu *et al.*.

The reaction is usually performed in methanol; however, other alcohols (ethanol and *n*-butanol) could also be used. Using this modified recipe, the 5-(4-(1*H*-imidazol-1-yl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (**30**) was obtained in 48% yield after the purification by flash chromatography (Scheme 33).

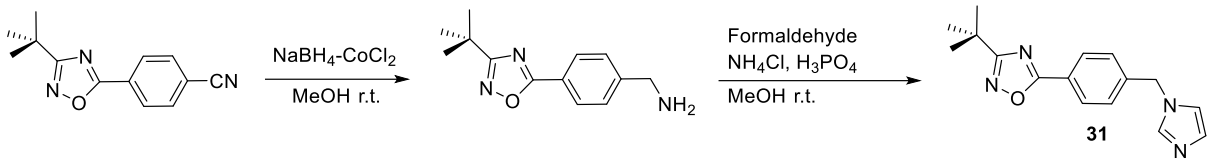


Scheme 33. Synthesis of 5-(4-(1*H*-imidazol-1-yl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (**30**).

The ^{1}H NMR spectrum ($CDCl_3$) displays the typical resonances corresponding to the imidazole moitey at 7.97 (1 H) ppm, 7.40 (1 H) ppm and 7.27 (1 H) ppm. The mass spectra show m/z = 268.1 (M^+), the loss of a methyl group (M-15). In the ^{13}C NMR spectrum the specific signals of the imidazole unit are found at 135.96 ppm, 131.81 ppm and 118.29 ppm.

III.3.2.3. Synthesis of 5-(4-((1*H*-imidazol-1-yl)methyl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (31)

The benzylic imidazole 5-(4-((1*H*-imidazol-1-yl)methyl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole was synthesized starting from the 1,2,4-oxadiazole-nitrile compound **5**. In a first step the benzylic amine ((4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)methanamine) was generated by reducing the nitrile functionality[201] and then, using the same procedure as for the phenyl type, the new benzyl imidazole derivative (**31**) was isolated (Scheme 34).



Scheme 34. Synthesis of 5-(4-((1*H*-imidazol-1-yl)methyl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (**31**).

The final yield after purification was low (30%). One of the reasons for the lower yield could be that the amine precursor was not pure enough. Despite several attempts only a purity of 75% was achieved. The spectral data shows in the ^{1}H NMR spectrum the aromatic protons (two at 8.10 ppm and two at 7.26 ppm), the imidazole signals at 7.58, 7.14 and 6.92 ppm (each for one proton), two singlet attributed to the methylene bridge at 5.21 (two protons) and at 1.43 ppm for the nine protons of the *t*-butyl group. The mass spectra has the molecular peak M^{+} = 282.2. Crystals suitable for X-ray diffraction analysis were obtained by slow evaporation of a methanolic solution of **31**.

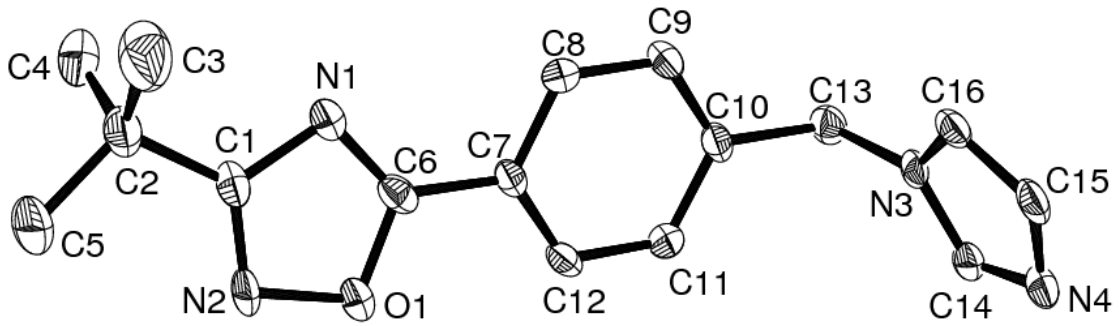


Figure 49. Molecular structure of 5-(4-((1*H*-imidazol-1-yl)methyl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (**31**). Atoms are drawn as 50% thermal ellipsoids. Selected bond lengths [Å] and angles [°]: O1-C6 1.356(3), O1-N2 1.422(2), N1-C6 1.297(3), N1-C1 1.392(3), N2-C1 1.304(3), N3-C14 1.341(3), N3-C16 1.369(3), N3-C13 1.466(3), N4-C14 1.321(3), N4-C15 1.377(3), C10-C13-N3 111.50(19).

The solid-state structure of **31** was established by X-ray diffraction analysis and shown in Figure 49 and selected bond distances and angles are listed in the figure caption. The compound crystallizes in the triclinic space group *P*-1 with two molecules in the asymmetric unit, which differ in the relative orientation of the rings.

III.3.3. Synthesis of 1,2,4-oxadiazole derivatives bearing 2-oxazoline moiety

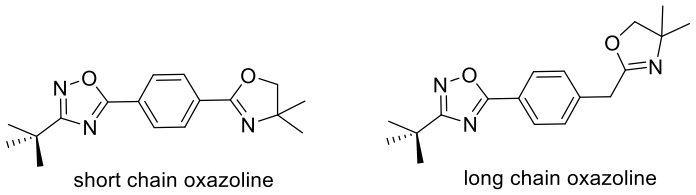


Figure 50. 1,2,4-Oxadiazoles bearing 2-oxazoline moiety as target molecules.

III.3.3.1. Background

Another substituent that was inserted into the 1,2,4-oxadiazole-containing molecules is the 2-oxazoline system, which is a five-membered heterocycle with significant applications in the synthesis of organic compounds[202] and in the field of drug discovery and development.[203,204] These bioactive molecules are usually generated by heating *N*-acyl derivatives of *β*-hydroxylamines, or by reacting them with thionyl chloride, sulfuric acid, or phosphorus pentoxide.[205] They can also be prepared by condensation of carboxylic acids with *β*-hydroxylamines at high temperatures under strongly acidic conditions.[204] Other synthetic protocols include the use of imino-ether hydrochlorides, nitriles and isocyanides.[205] Vorbrüggen *et al.*[204] reported a one-pot synthesis starting from readily available carboxylic acids using Ph_3P/CCl_4 as an activating agent. Finally, carboxylate esters can be directly transformed into 2-oxazolines using lanthanum chloride as catalyst.[206]

This class of heterocyclic compounds has received much attention, since many compounds carrying a 2-oxazoline motif have biological activity (Figure 51).

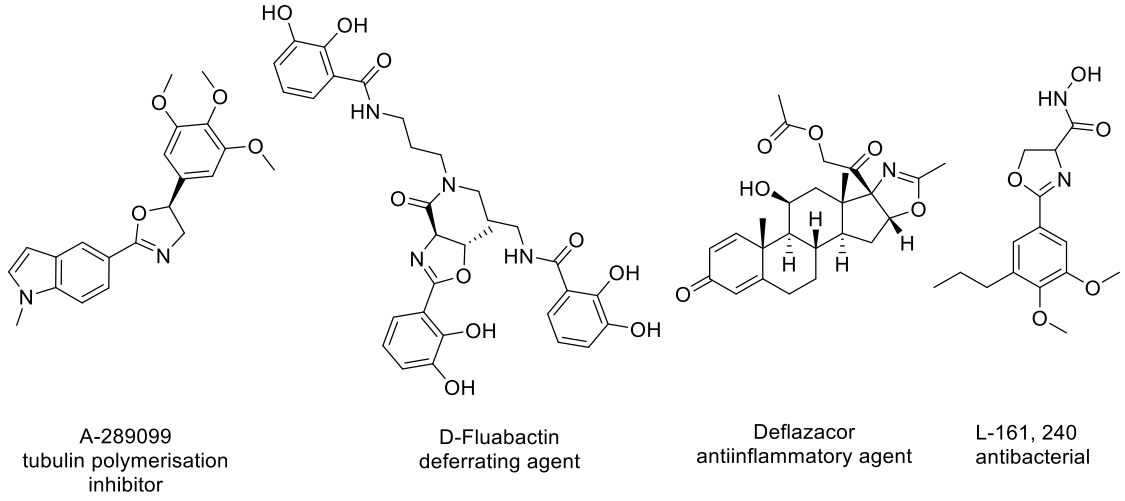


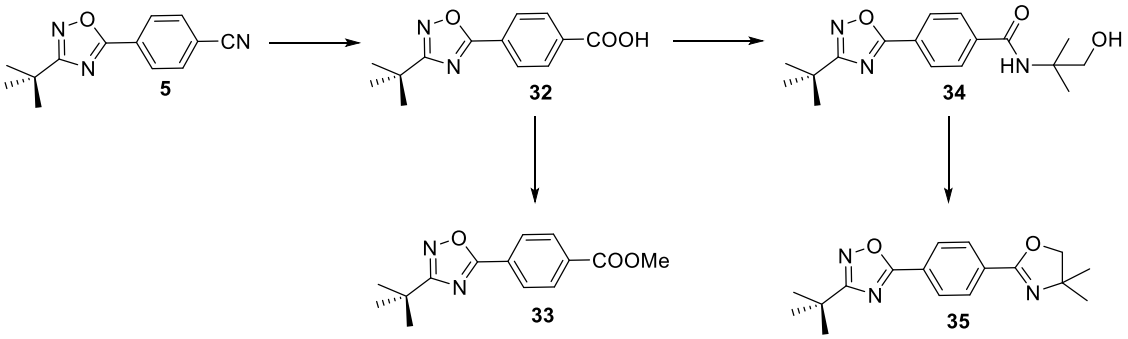
Figure 51. Biologically active compounds containing the 2-oxazoline moiety.

2-Indolyloxazolines are potential candidates as oral anticancer agents because of their potent inhibition of tubulin polymerization.[207] Another example is D-fluviabactin, which, in

a model of iron overload in chronically transfused thalassemia patients, has been shown to efficiently sequester and remove iron from animals.[208] Deflazacor (commercially available as Dezacor, Flantadin and Lantadin) is a corticosteroid derivative used as an inflammatory agent.[209] L-161, 240 has been reported as a strong antibacterial agent, with a minimal inhibitory concentration comparable to those of other clinically relevant antibiotics, such as Ampicillin or Rifampicin.[210] In addition, 2-oxazolines have also been described as potential prodrug precursors of carboxylic acids.[211]

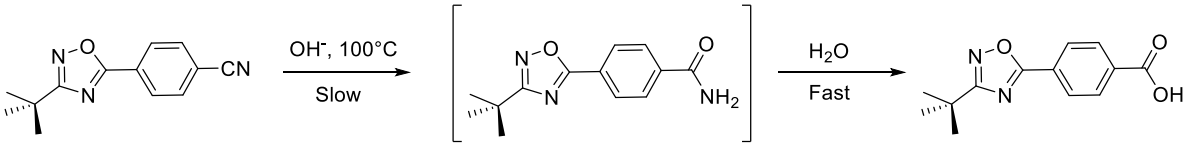
III.3.3.2. Synthesis of 3-(*tert*-butyl)-5-(4-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)phenyl)-1,2,4-oxadiazole (35)

Starting from 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzonitrile **5** it was planned to generate 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzoic acid (**32**). The acid derivative is one of the intermediates in the synthesis of the main target product, 3-(*tert*-butyl)-5-(4-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)phenyl)-1,2,4-oxadiazole (**35**). Scheme 35 shows the general approach for the synthesis of **35**.



Scheme 35. General synthetic route for 3-(*tert*-butyl)-5-(4-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)phenyl)-1,2,4-oxadiazole (**35**).

The hydrolysis of nitriles is one of the main synthetic routes for building amides and carboxylic acids.[212] Because of the limited reactivity of nitriles, their transformation in most cases requires harsh reaction conditions, such as strongly acidic[213] or basic,[214] generating as intermediate the corresponding amide, which in most cases is hydrolyzed (Scheme 36).



Scheme 36. Hydrolysis of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzonitrile in basic conditions.

From the carboxylic acid **32**, the methyl ester derivative, methyl 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzoate (**33**), was generated rapidly and quantitatively by reaction with diazomethane (1 *N* in diethyl ether). The solid-state structure of **33** was established by X-ray diffraction analysis and is shown in Figure 52. Similarly to **5**, **33** crystallized in the monoclinic space group $P2_1/m$ with imposed mirror symmetry, although the ester group is slightly disordered to both sides of the mirror plane.

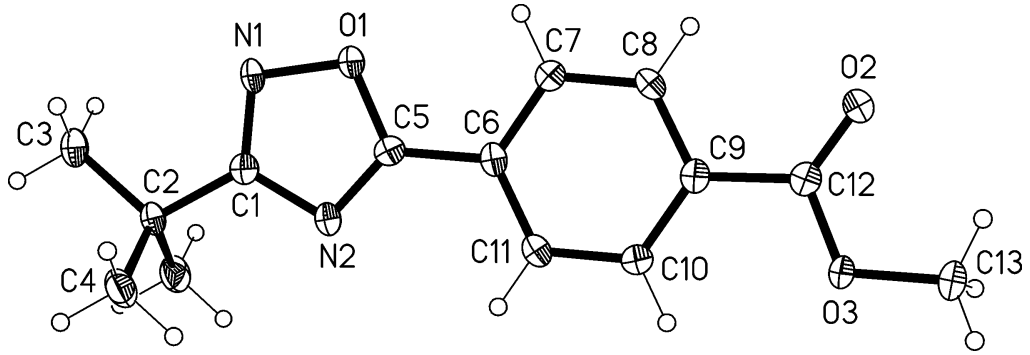
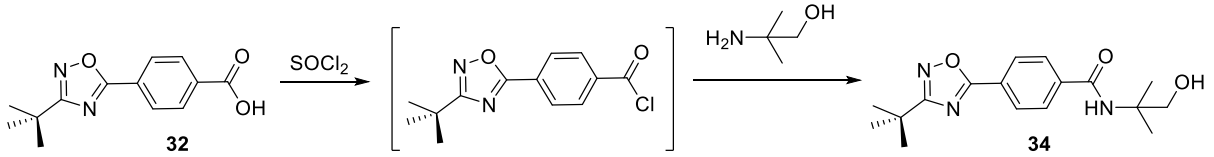


Figure 52. Molecular structure of methyl 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)benzoate (**33**). Only one position of the disordered ester group is shown. Atoms are drawn as 50% thermal ellipsoids. Selected bond lengths [Å] : N1-C1 1.309(2), N2-C1 1.389(2), O1-N1 1.428(2), O1-C5 1.346(2), N2-C5 1.299(2), O(2)-C(12) 1.207(2), O3-C12 1.346(3), O3-C13 1.452(3).

In order to introduce the oxazoline motif into the 1,2,4-oxadiazol system, we first had to generate the amide intermediate 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)-N-(1-hydroxy-2-methylpropan-2-yl)benzamide (**34**) from the acid **32** (Scheme 37). The acyl chloride derivative was generated in situ by reacting the acid with $SOCl_2$ at room temperature and used further (without purification, as a solution in DCM) in the highly exothermic reaction with 2-amino-2-methyl-1-propanol at low temperature. Control of the temperature is crucial because at temperatures above 0°C several byproducts are generated and the purification is more difficult.



Scheme 37. Synthesis of the amido derivative **34** via the acyl chloride route.

The amide **34** was cyclized to the corresponding oxazoline **35** by the dropwise addition of thionyl chloride at 0 °C. After treatment with aqueous NaOH and extraction with diethyl ether, the oxazoline **35** was isolated as a colorless solid in good yield (86%). The solid-state structure of **35** was established by X-ray diffraction analysis (Figure 53). The compound crystallizes without imposed symmetry in the monoclinic space group $P2_1/c$. The

ring systems display typical bond lengths and angles. The oxadiazole and the central ring are essentially parallel (interplanar angle 1(15)°), whereas the oxazole ring is rotated by 11.5(13)° with respect to the central ring. This rotation is sufficiently small that the molecule still displays approximate mirror symmetry (r.m.s. deviation 0.2 Å).

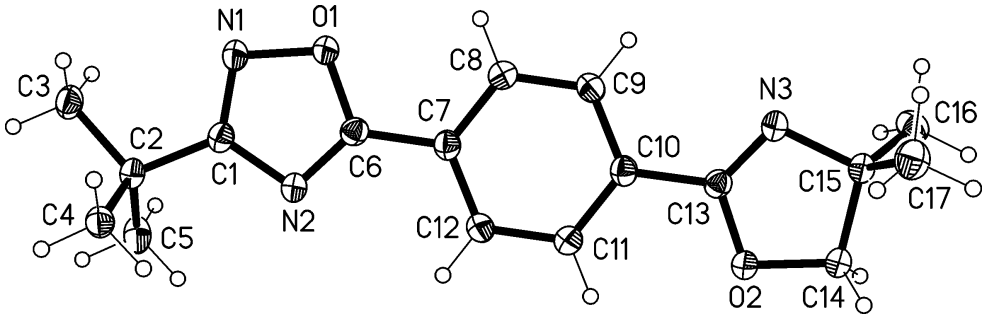
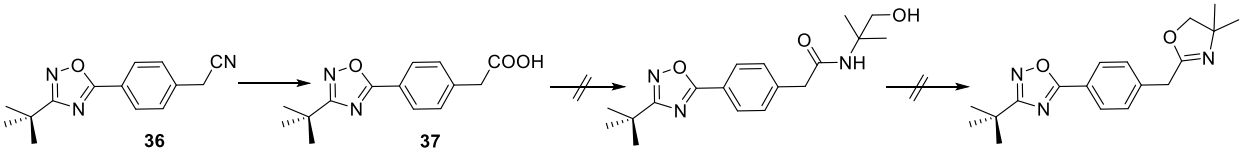


Figure 53. Molecular structure of 3-(*tert*-butyl)-5-(4-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)phenyl)-1,2,4-oxadiazole (**35**). Atoms are drawn as 50% thermal ellipsoids. Selected bond lengths [Å]: N1-C1 1.3039(14), N2-C1 1.3876(12), O1-N1 1.4194(11), O1-C6 1.3453(12), N2-C6 1.2982(13), O2-C13 1.3621(12), O2-C14 1.4498(12), N3-C13 1.2680(14), N3-C15 1.4834(12).

III.3.3.3. Attempt for the synthesis of 3-(*tert*-butyl)-5-(4-((4,4-dimethyl-4,5-dihydrooxazol-2-yl)methyl)phenyl)-1,2,4-oxadiazole

In our attempt to synthesize 3-(*tert*-butyl)-5-(4-((4,4-dimethyl-4,5-dihydrooxazol-2-yl)methyl)phenyl)-1,2,4-oxadiazole, a longer chain oxazoline derivative, we failed to generate the amide intermediate (Scheme 38). In a first step the cyanide compound 2-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)acetonitrile **36** was prepared from 4-(cyanomethyl)benzoic acid. The reaction followed the general synthetic protocol but the yield and purity for **36** were dramatically lower. All attempts to purify the compound failed and I was forced to use it despite 40% impurities.



Scheme 38. Proposed synthetic route for 3-(*tert*-butyl)-5-(4-((4,4-dimethyl-4,5-dihydrooxazol-2-yl)methyl)-phenyl)-1,2,4-oxadiazole.

It was possible to synthesize and isolate the acid derivative 2-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)acetic acid **37** using the same saponification method as for compound **32**. The solid-state structure of **37** was established by X-ray diffraction analysis and shown in Figure 54. The compound crystallizes in the orthorhombic space group *Pbcn* without imposed symmetry. Molecules are connected by the well-known carboxylic acid dimer motif across inversion centers.

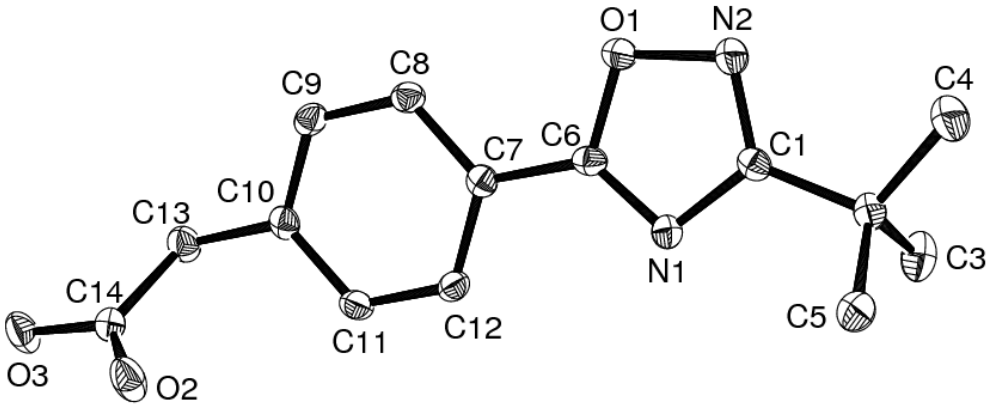


Figure 54. Molecular structure of ethyl 2-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)acetic acid (**37**). Atoms are drawn as 50% thermal ellipsoids. Selected bond lengths [Å] : N1-C1 1.3840(14), N2-C1 1.3038(13), O1-N2 1.4256(11), O1-C6 1.3520(12), N1-C6 1.3002(13), O2-C14 1.2159(13), O3-C14 1.3195(13).

The formation of the amide derivative in the next step was not successful. The reaction was repeated 3 times with minor modifications of the protocol (lower temperature, shorter time), but all attempts failed. The reaction mixture changed color from colorless to black and

no signs of the amide derivative were detected. Other synthetic routes are currently still under investigation.

III.3.3.4. *In vitro* anti-tumor activity towards human tumor cell lines of 1,2,4-oxadiazole bearing oxazoline and imidazole moieties.

In vitro anti-tumor activity of three compounds was assessed using a monolayer cell survival and proliferation assay in a panel of 11 cell lines, comprising colon, gastric, lung, ovarian, pancreatic, prostate, renal and uterus cancer, as well as melanoma. Concentration-dependent activity was detected for compound **35** across all cell lines tested. By exhibiting a geometric mean IC_{50} value of 17. µM (Figure 55), IC_{50} values for the individual cell lines were in the range from 11.9 µM (UXF 1138, uterus cancer) and 24.2 µM (MEXF 462, melanoma). Overall, good potency was found for the oxazoline derivative **35** towards all cell lines investigated. The other two imidazole derivatives **30** and **31**, with a mean IC_{50} value of 43.9 µM and 54.9 µM respectively, have only moderate anti-tumor activity towards the 12 cell lines tested.

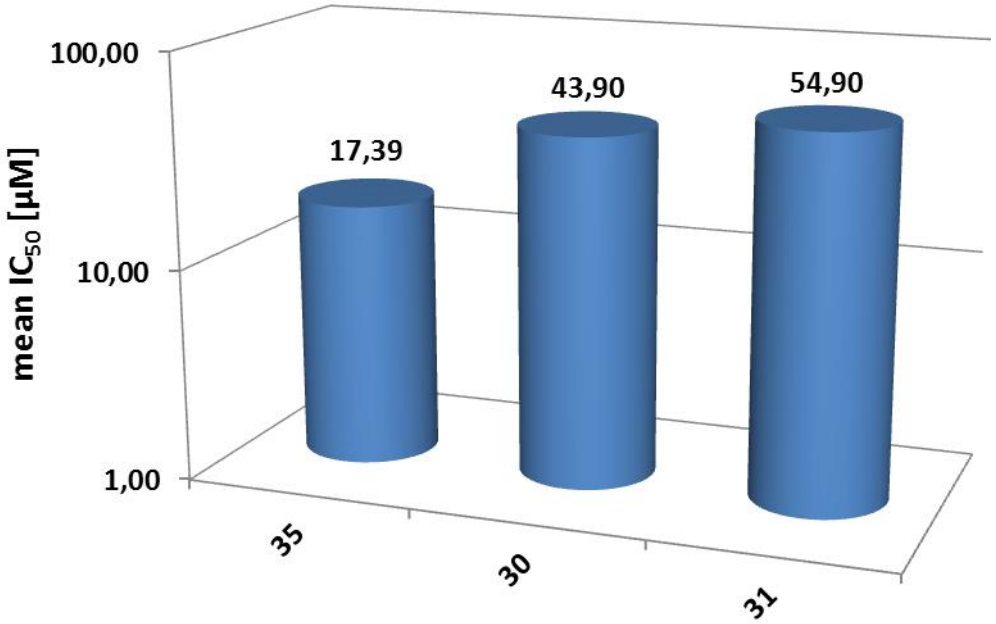


Figure 55. In vitro anti-tumor activity of compounds **30**, **31** and **35** towards 11 human tumor cell lines.

In Figure 56 the tumor selectivity of compounds **30, 31** and **35** is illustrated. Although the oxazoline compound has good activity, it shows no selectivity. Nevertheless, the two imidazole derivatives **30** and **31** have just moderate activity, but they show some promising selectivity. Compound **30** is selective towards MAFX 401 cell line (mammary tumor cell), whereas its analogue **31** with a longer chain proved to have good selectivity against UXF 1138 cell line (uterus tumor cell).

compound	unit	Cell lines											Geom. mean IC_{50} [μM]
		CXF HT-29	GXF 251	LXFA 629	LXFL 529	MAXF 401	MEXF 462	OVXF 899	PAXF 1657	PXF 1752	RXF 486	UXF 1138	
35	μM	13,60	15,40	14,70	15,70	16,90	24,20	21,90	23,60	20,60	9,27	22,60	**17,39**
30	μM	100,0	63,6	49,8	23,3	11,0	100,0	28,6	100,0	58,97	36,01	23,58	**43,9**
31	μM	53,0	31,7	59,4	31,9	33,1	100,0	85,3	100,0	68,42	96,70	22,80	**54,9**

1/32 1/16 1/8 1/4 1/2 1 2 4 8 16 32 -fold mean IC_{50}

sensitive cell lines — resistant cell lines

Figure 56. Individual IC_{50} values [μM] of compounds **30**, **31** and **35** in a panel of 11 human tumor cell lines.

III.3.4. Synthesis of 1,2,4-oxadiazole derivatives bearing pyrazol-pyrimidines moiety

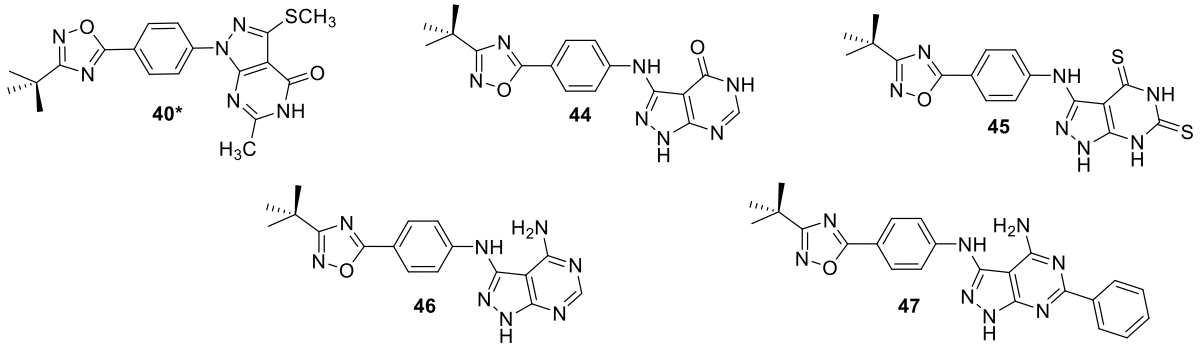


Figure 57. Target 1,2,4-oxadiazoles bearing pyrazol-pyrimidine moiety.

III.3.4.1. Background

Pyrazol-pyrimidines and related fused heterocycles are bioactive and act as central nervous system depressants,[215] narcoleptic agents,[216] anti-tuberculosis agents,[217] adenosine receptors[218] and as powerful antitumor agents.[219-221] Along with indolocarbazoles and 2-indolinone derivatives, these heterocycles act as inhibitors for RET (REarranged during Transfection).[222-225] Alternatively pyrazole-pyrimidine-based compounds can also be used as scaffolds in the inhibition of the PFPK7 kinase of *Plasmodium falciparum*.[226] Additionally, the pyrazole-pyrimidine derivative **A** is a good inhibitor of many tyrosine kinases (TKs) in cancer treatment,[227] whereas compound **B** is a promising candidate as an RET protein kinase inhibitor.[228] Medley *et al.* demonstrated that **C** is strong covalent inhibitor of interleukin-2 inducible T cell kinase (Itk) (Fig. 58).[229]

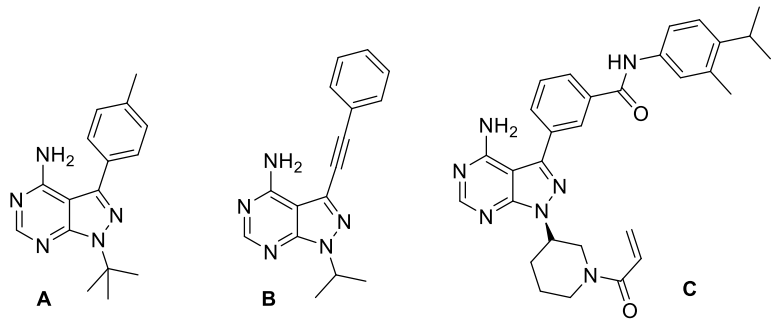
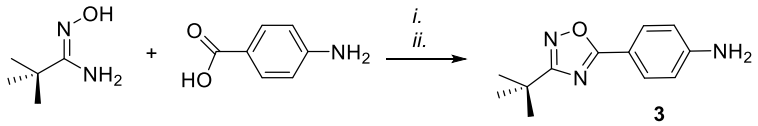


Figure 58. Examples of bioactive pyrazol-pyrimidines derivatives.

The plan was to attach the pharmacologically active 1,2,4-oxadiazole-substituted phenyl to the pyrazole moiety of pyrazole-pyrimidines.[230]

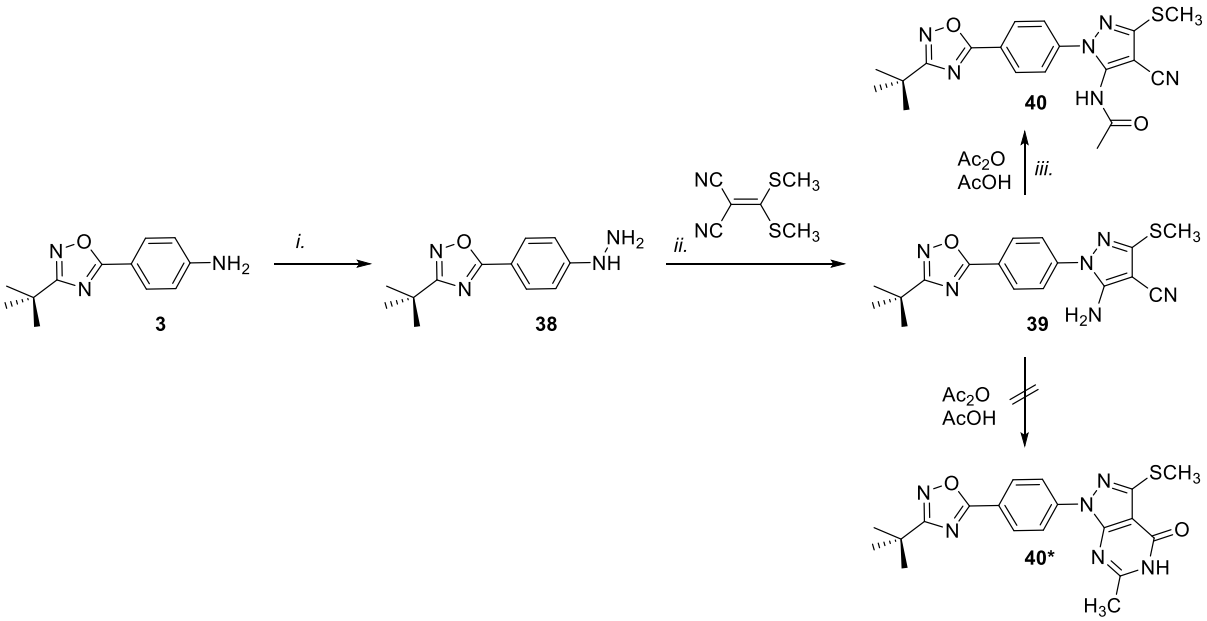
In previous work it was reported the synthesis of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)aniline (**3**) as a precursor to several natural product analogues, which were tested *in vitro* for antitumor activity toward a panel of 11 cell lines using a monolayer cell survival and proliferation assay.[231,232]



Scheme 39. One pot synthesis of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)aniline (**3**) using the amidoxime route: *i.* 1,1 eq. CDI in DMF, 30 minutes; *ii*. 1,1 eq. CDI in DMF, 120°C, 4h.

III.3.4.2. Attempt for the synthesis of 1-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-6-methyl-3-(methylthio)-1,5-dihydro-4*H*-pyrazolo-[3,4-d]pyrimidin-4-one (40*)

Using the amine **3** as starting points, the idea was to construct pyrazole-pyrimidine derivatives bearing 1,2,4-oxadiazole unit to increase their antitumor activity. One of the first synthetic targets was 1-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-6-methyl-3-(methylthio)-1,5-dihydro-4*H*-pyrazolo-[3,4-d]pyrimidin-4-one (**40***). The first step was the transformation of amine **3** to the corresponding hydrazine derivative 3-(*tert*-butyl)-5-(4-hydrazinylphenyl)-1,2,4-oxadiazole (**38**) using known procedures (Scheme 40).[233,234]



Scheme 40. Synthetic route to compounds **38**, **39** and **40**. Reagents and conditions: *i*. $NaNO_2$, H_2SO_4, $SnCl_2$, HCl, 0 °C; *ii*. MeOH, reflux; *iii*. Ac_2O, AcOH, reflux.

After the successful isolation of the hydrazine derivative, the next step was the reaction with 2-(bis(methylthio)methylene)malononitrile under reflux in methanol in order to generate 5-amino-1-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-3-(methylthio)-1*H*-pyrazole-4-carboni-trile (**39**). The structure of **39** was confirmed by an X-ray structure determination (Figs. 59 and 59a). It crystallizes in the monoclinic space group *C*2/*c*. Bond lengths and angles are unexceptional, as is the case for all other structures reported here. The central ring subtends an interplanar angle of 19(3)° to the oxadiazole ring and 28(2)° (in the same direction) to the pyrazole ring (for which all substituents also lie in the ring plane). The hydrogens of the NH_2 group are involved in classical hydrogen bonds H02ΛN13 (short and linear) and H01ΛN2 (longer and rather angled, probably attributable to the steric hindrance of the central ring), leading to ribbons of H-bonded rings parallel to [112].

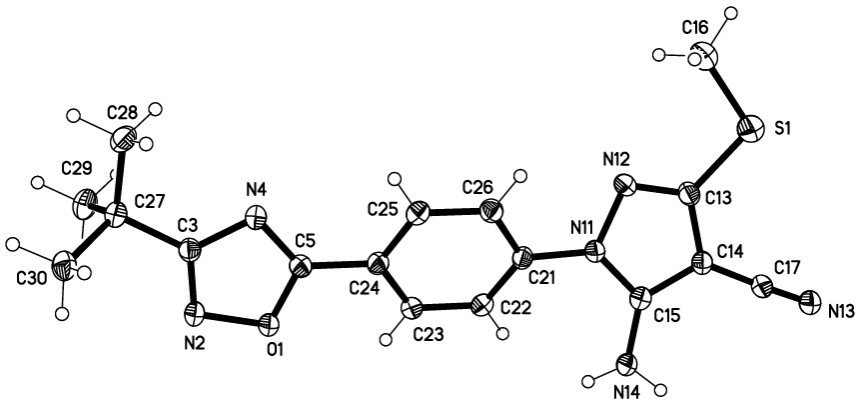


Figure 59. Molecular structure of 5-amino-1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(methylthio)-1*H*-pyrazole-4-carbonitrile (**39**). Atoms are drawn as 50% thermal ellipsoids.

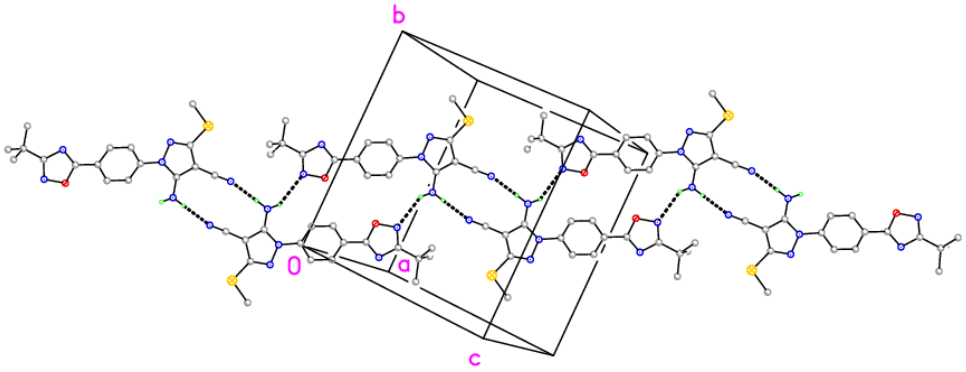


Figure 59a. Packing diagram of compound **39**. Hydrogen bonds are indicated as dashed lines.

The final step was the cyclisation of the pyrazole derivative **32** in the presence of Ac_2O and AcOH at reflux using reported procedures.[235] However, the cyclisation to the desired product (**40*)** failed. The reaction was repeated twice, and in each case, the product was *N*-(1-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-4-cyano-3-(methylthio)-1*H*-pyrazol-5-yl)-acetamide (**40**). Derivatives **40** and **40*** can easily be confused, since they have the same exact mass and give rise to similar NMR signals. However, I succeeded in growing crystals from the isolated product, and in our case, the final product is indeed **40**. With this result in hand, I decided to abandon this route of building novel pyrazole-pyrimidines and to pursue other synthetic strategies.

The solid-state structure of **40** was established by X-ray diffraction analysis and is shown in Figure 60. Compound **40** crystallizes in the monoclinic space group $P2_1/n$. The three rings adopt a similar arrangement as in **39**, with the central ring subtending an angle of 20(2)° to the oxadiazole and 23(3)° to the pyrazole ring (so that the five-membered rings are almost mutually parallel). The molecules are linked to form chains parallel to the *a* axis by the classical hydrogen bond H01ΛO2; the amide group is thus both donor and acceptor (Fig. 60a).

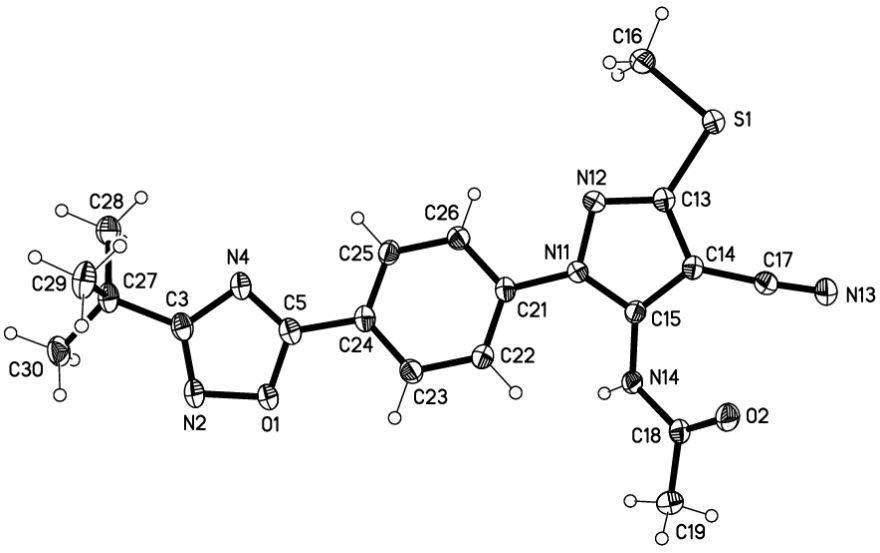


Figure 60. Molecular structure of *N*-(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl) phenyl)-4-cyano-3-(methylthio)-1*H*-pyrazol-5-yl)acetamide (**40**). Atoms are drawn as 50% thermal ellipsoids.

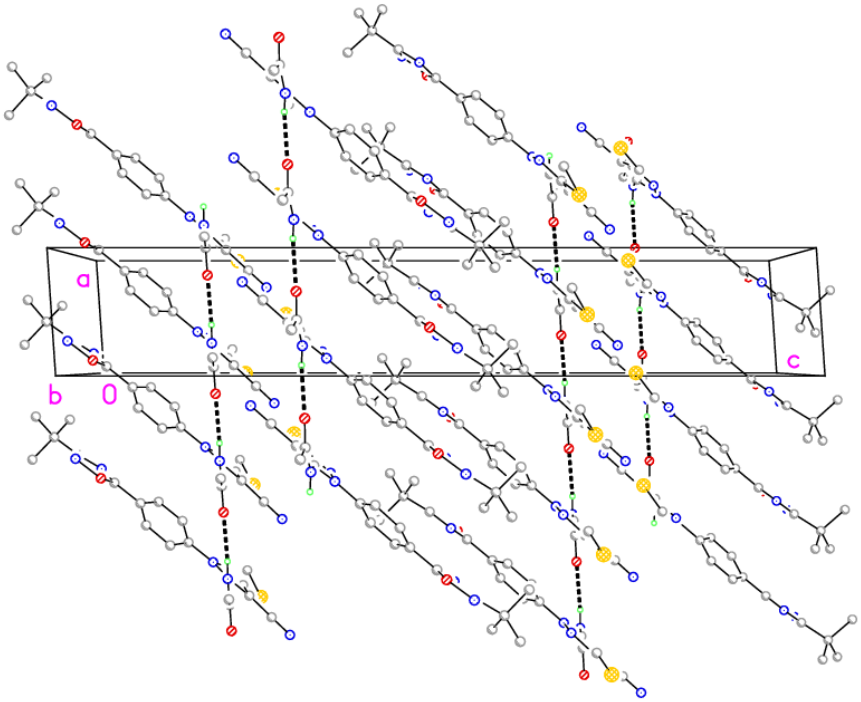
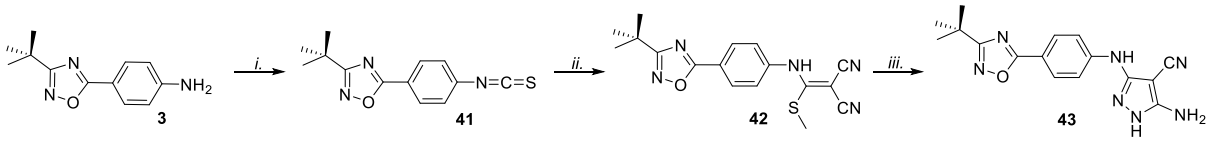


Figure 60a. Packing diagram of compound **40**. Hydrogen bonds are indicated as dashed lines.

III.3.4.3. Synthesis of the key intermediate 5-amino-3-((4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)amino)-1*H*-pyrazole-4-carbonitrile (43)

The key intermediate **37** was accessed using a previously described general method for generating pyrazole derivatives (Scheme 41).[236,237]



Scheme 41. Synthesis of the 5-amino-4-cyano-1*H*-pyrazole key intermediate **43**. Reagents and conditions: *i.* Na_2CO_3, Thiophosgene, H_2O/dichloro-methane, 0 °C – r.t.; *ii.* Malononitrile, CH_3I, DMF, NaH, 0 °C – r.t. – 55ºC; *iii.* Hydrazine, MeOH, reflux.

Starting from the available amine derivatives **3**, the corresponding isothiocyanates **41** (3-(*tert*-butyl)-5-(4-isothiocyanatophenyl)-1,2,4-oxadiazole) was formed in excellent yields (96%) and good purity. The structure of **41** was also confirmed by X-ray structure analysis (space group $P2_1/n$; Figs. 61, 61a); the two rings along with the isothiocyanate group are co-planar (mean deviation 0.02 Å). The thiocyanate group displays bond lengths N11-C17 and C17-S1 of 1.1720(17) Å and 1.5752(13) Å, respectively, and it is also aprroximately linear, with angles C17-N11-C14 162.74(14)° and N11-C17-S1 175.98(12)°. In the absence of classical H bond donors, the molecules are linked by the "weak" interactions H13ΛO1, N2 (three-centre) and H16ΛS to form ribbons parallel to [101].

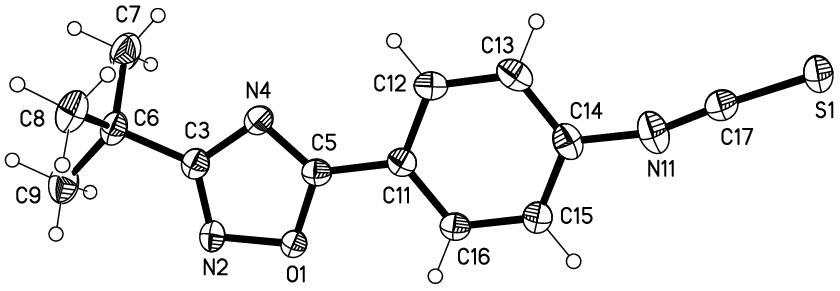


Figure 61. Molecular structure of 3-*tert*-butyl-5-(4-isothiocyanatophenyl)-1,2,4-oxadiazole (**41**). Atoms are drawn as 50% thermal ellipsoids.

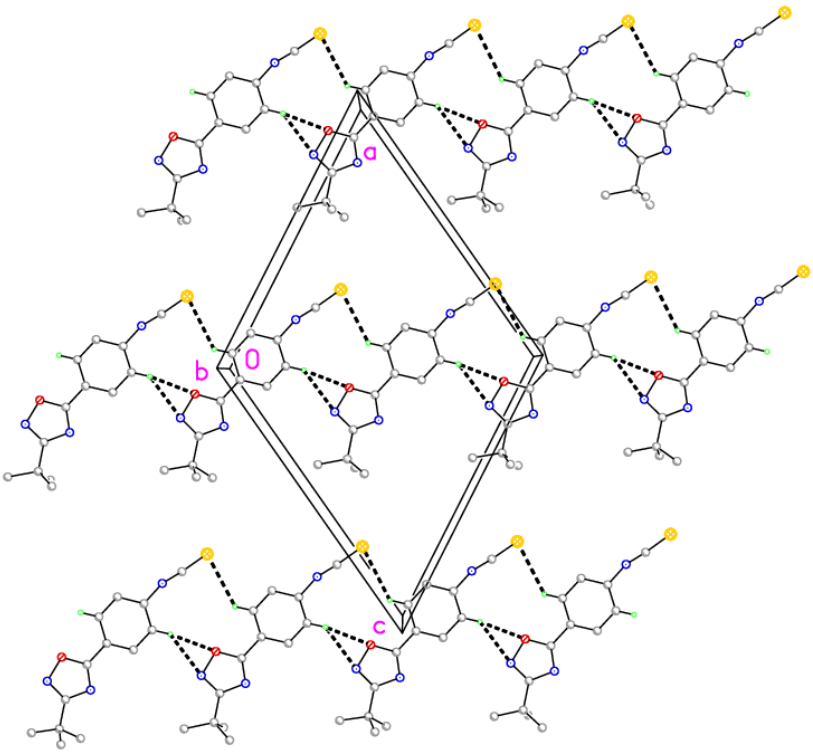


Figure 61a. Packing diagram of compound **41**. Hydrogen bonds are indicated as dashed lines.

Further treatment of **41** with malononitrile, in the presence of NaH and CH_3I, afforded 2-(((4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl) amino)-(methylthio)methylene)-malononitrile (**42**). The molecular structure of **42** was also established by X-ray crystallography. In compound **42** (Figs. 62, 62a), the oxadiazole and the phenyl ring are approximately co-planar. The molecule crystallized in space group $P\bar{1}$ with two molecules in the asymmetric unit, which differ slightly in the relative orientation of the rings; a least-squares fit gave an r.m.s. deviation of 0.10 Å (excluding *t*-butyl groups). The NH hydrogens are involved in hydrogen bonds H01ΛN12' and H01'ΛN12, thereby connecting the molecules by translation to form chains parallel to the *y* axis.

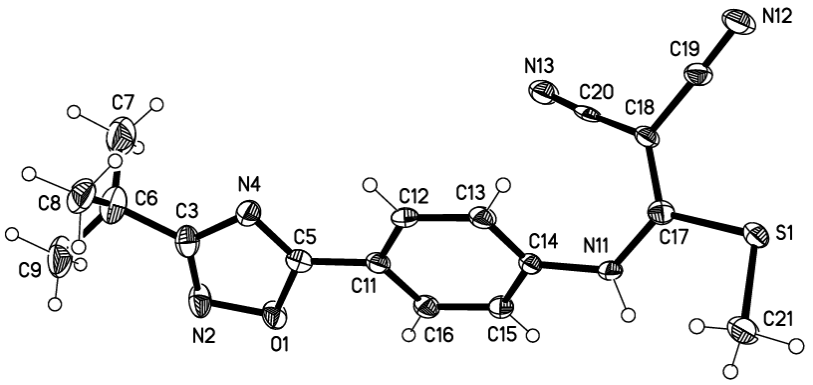


Figure 62. Molecular structure of 2-((4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl) phenylamino)-(methylthio)methylene) malononitrile (**42**). Only one of the two independent molecules is shown, with only one position of the disordered *t*-butyl group. Atoms are drawn as 50% thermal ellipsoids.

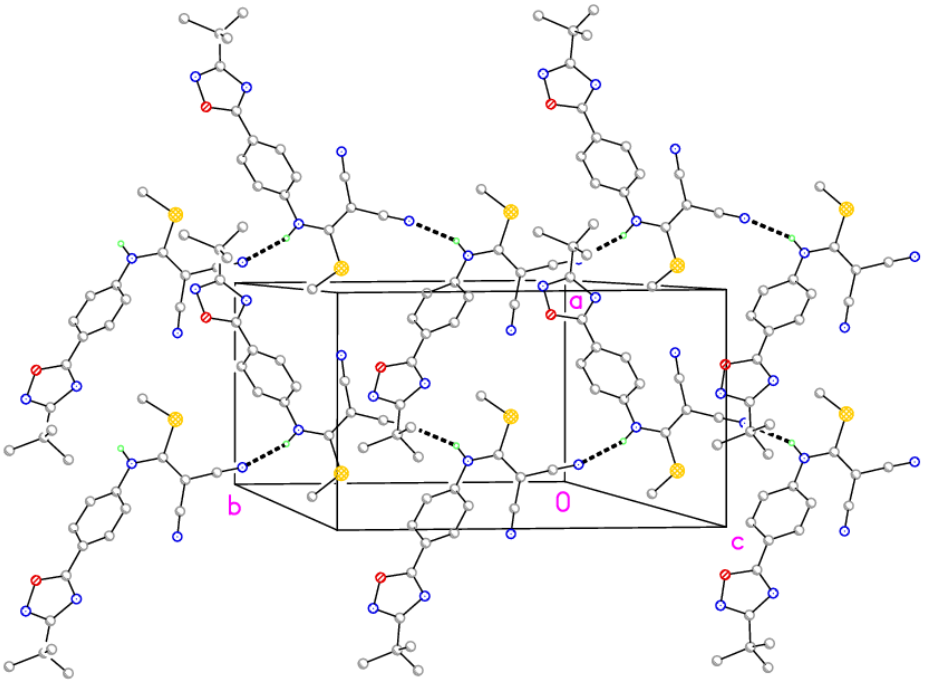


Figure 62a. Packing diagram of compound **42**. Hydrogen bonds are indicated as dashed lines.

Cyclisation of **42** with hydrazine monohydrate in refluxing methanol gave the pyrazoles **43** in excellent yields (93%). The solid-state structure of **43** was established by X-ray diffraction analysis (Fig. 63). The compound crystallizes as a methanol solvate in the space group $P2_1/n$. The central ring subtends angles of 19(18)° and 16(2)° to the oxadiazole and pyrazole rings respectively. Within the asymmetric unit, the methanol forms a hydrogen bond O–HΛN12. There are four further hydrogen bond donors (NH groups) in the molecule of **43**, and these are all involved in hydrogen bonding; the net result is to link the molecules to form highly corrugated sheets perpendicular to the *b* axis (Fig. 63a).

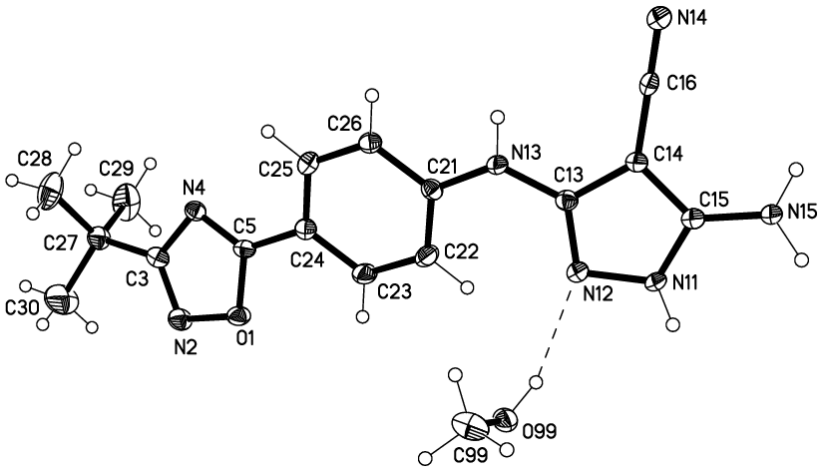


Figure 63. Molecular structure of 5-amino-3-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylamino)-1*H*-pyrazole-4-carbonitrile (**43**) as its methanol solvate. Atoms are drawn as 50% thermal ellipsoids. The dashed line indicates a hydrogen bond.

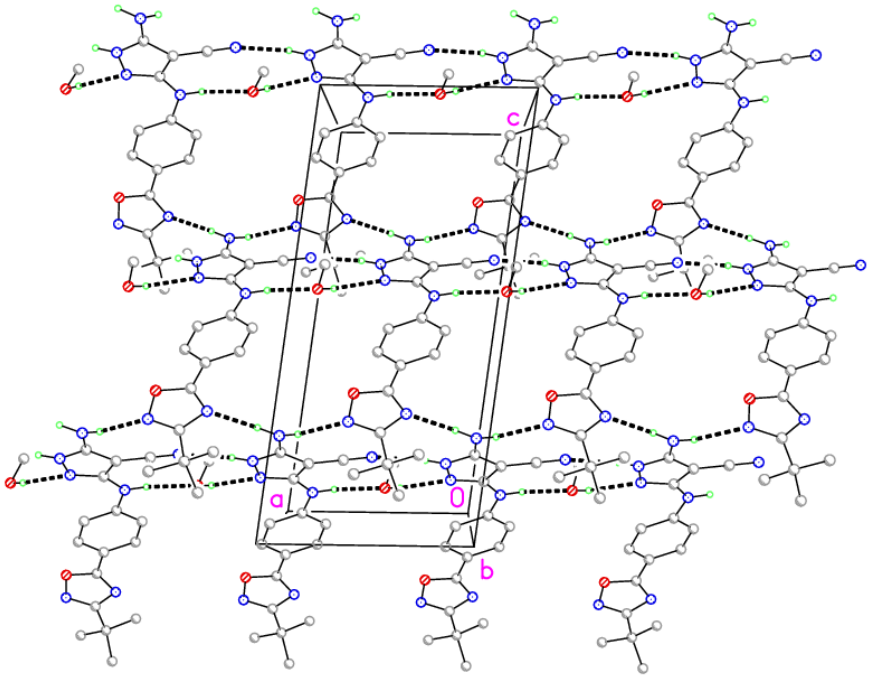
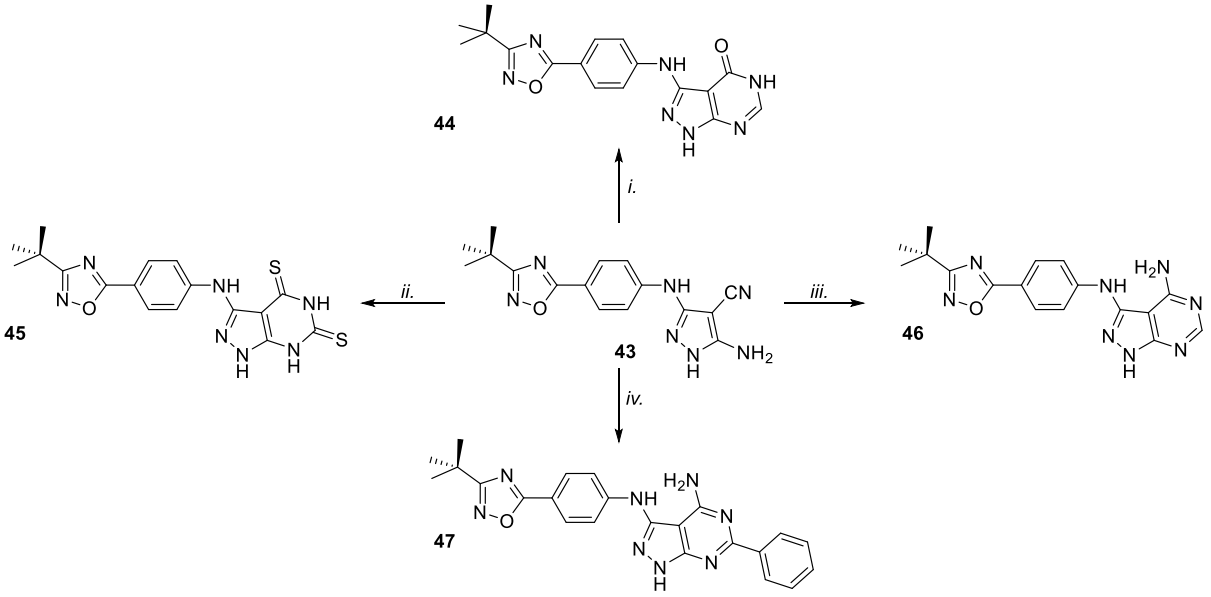


Figure 63a. Packing diagram of compound **43**. Hydrogen bonds are indicated as dashed lines.

III.3.4.4. Synthesis of N^3-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-6-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamine (47) and other pyrazolo[3,4-d]pyrimidine derivatives

After the generation of the pyrazol intermediate, the synthesis of several novel pyrazolo[3,4-*d*]pyrimidines bearing a 1,2,4-oxadiazole unit was readily achieved (Scheme 42).



Scheme 42. Synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives. Reagents and conditions: *i*. HCOOH, reflux 2 h; *ii*. CS_2, pyridine, reflux 24 h; *iii*. $HCONH_2$, reflux 5 h; *iv*. Benzamidine, NaOAc, Naphthalene, 230°C 1 h.

3-((4-(3-(*tert*-Butyl)-1,2,4-oxadiazol-5-yl)phenyl)amino)-1,5-dihydro-4*H*-pyrazolo-[3,4-*d*]pyrimidin-4-one (**44**) was obtained in moderate yield (63%) by refluxing compound **43** in formic acid for several hours. When the formic acid was replaced by CS_2 in dry pyridine, 3-((4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)amino)-1,7-dihydro-4*H*-pyrazolo[3,4-*d*] pyrimidine-4,6-(5*H*)-dithione (**45**) was isolated, but only in low yield (16%). When formamide was employed as an alternative cyclisation reagent, the pyrazol derivative **43** was converted after heating at reflux for 5 h to N^3-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamine (**46**).

In order to construct a more bulky derivative bearing one aromatic ring directly connected to the pyrazol-pyrimidine core, it was used benzamidine hydrochloride as a cyclization reagent. However, high temperatures are required in order to achieve ring closure. All the attempts at generating N^3-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-6-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamine (**47**) using the usual organic solvents with high boiling points (120-150°C) produced only trace amounts of the desired product. To increase the reaction temperature, I tried to perform this reaction without solvents at 200°C.

Unfortunately, decomposition occurred within 5 min (the mixture turned black) and the desired products were only isolatedin very low yield. This problem was solved by using naphthalene as the solvent; after 30 min at 220°C, the desired compounds were isolated.

The solid-state structure of **47** was determined by X-ray analysis (Figs. 64 and 64a). It crystallizes with three independent molecules and five DMSO in the asymmetric unit. The molecules of **47** are very approximately planar (except for the *t*-butyl groups), but the rings are mutually rotated in a manner that differs significantly in the three molecules (e.g. in molecule *2*, the ring C31-C36 is much more strongly rotated with respect to the other rings). The packing is at first sight complex, but the overall scheme is clear; two complete formula units form an aggregate with overall inversion symmetry. In the centre of the aggregate, two molecules *3* are connected by hydrogen bonds N1"–H01"ΛN7". Molecule *3* is connected to molecule *2* via DMSO *4*, with the hydrogen bonds N8"–H04"ΛO4 and N8'–H04'ΛO4. Molecule *2* is connected to molecule *1* by the hydrogen bonds N1'–H01'ΛN7 and N1–H01ΛN7'. Finally, the remaining DMSO molecules are hydrogen bonded at the periphery of the aggregate (DMSO *2* rather weakly, with N8–H04ΛO2 2.57 Å).

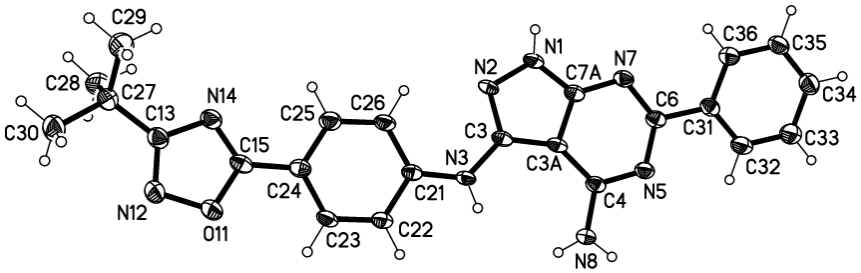


Figure 64. Molecular structure of N^3-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl) phenyl)-6-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamine (**47**); one of three independent molecules (solvent omitted) Atoms are drawn as 50% thermal ellipsoids.

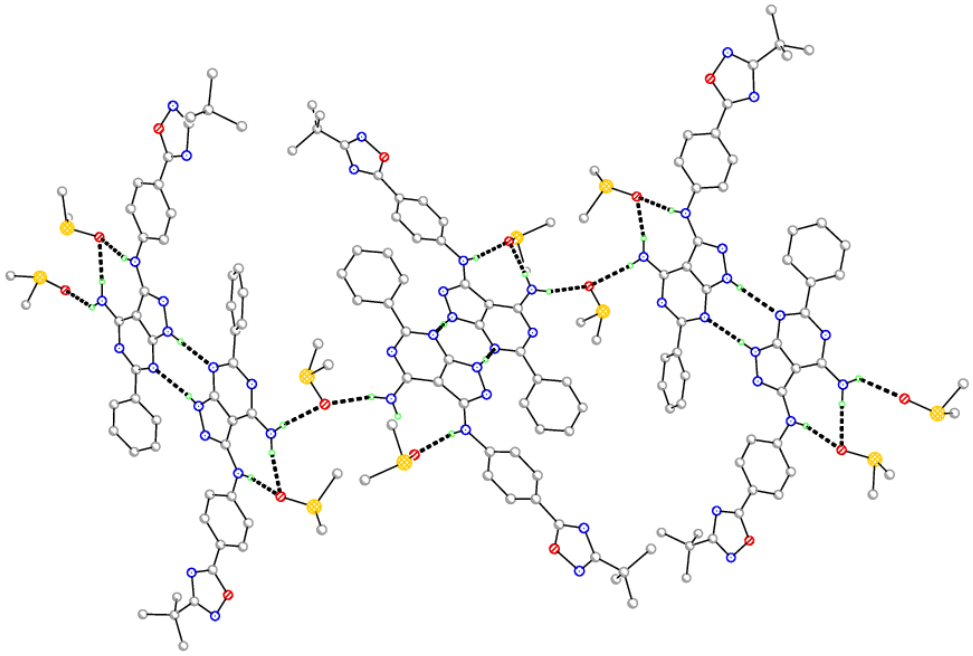


Figure 64a. Packing diagram of compound **47**. Hydrogen bonds are indicated as dashed lines.

III.3.4.5. *In vitro* anti-tumor activity towards human tumor cell lines of 1,2,4-oxadiazole derivatives bearing pyrazol-pyrimidines moiety and intermediates.

The *in vitro* anti-tumor activity of the compounds **39**, **43**-**47** was assessed in a panel of 12 human tumor cell lines by using monolayer cell survival and proliferation assays. As shown in Figure 65, good potency with a mean IC_{50} value of 5.66 µM was detected for compound **47**. Furthermore, at a lower level, anticancer activity was established for **43**, **46**, **44**, **39** and **45** with mean IC_{50} values in the range from 31.3 µM to 57.1 µM.

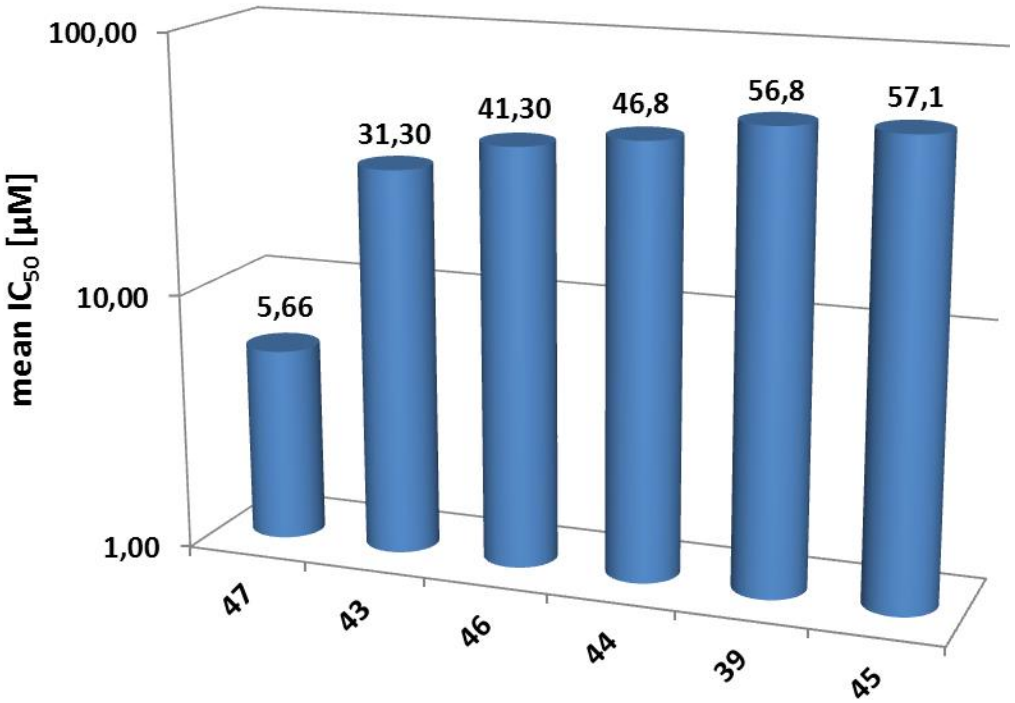


Figure 65. In vitro anti-tumor activity of compounds **39**, **43-47** in a panel of 12 human tumor cell lines (mean IC_{50} values).

Figure 66 shows the individual IC_{50} values as a heatmap presentation. The most active compound, **47**, displayed individual IC_{50} values in the range from 2.76 µM (OVXF 899) to 9.27 µM (PXF 1752). However, these compounds showed no pronounced selectivity for any of the tested cell lines.

compound	unit	Cell lines												Geom. mean IC_{50} [µM]
		CXF HT-29	GXF 251	LXFA 629	LXFL 529	MAXF 401	MEXF 462	OVXF 899	PAXF 1657	PRXF 22Rv1	PXF 1752	RXF 486	UXF 1138	
47	µM	2,90	5,49	6,89	5,94	4,54	9,66	2,76	7,83	5,60	9,27	7,07	4,81	**5,66**
43	µM	28,92	27,08	32,66	22,41	30,79	39,55	25,75	40,55	36,57	32,33	36,28	28,09	**31,28**
46	µM	45,16	45,54	36,07	39,62	44,17	44,48	44,39	58,20	41,57	37,55	41,52	25,28	**41,27**
44	µM	51,65	37,99	52,42	47,78	40,45	38,48	50,94	52,43	66,79	48,43	42,69	39,06	**46,79**
39	µM	17,34	100,0	14,85	32,20	28,67	100,0	100,0	100,0	100,0	100,0	100,0	47,29	**56,79**
45	µM	75,84	77,29	100,0	89,09	100,0	38,94	66,97	18,85	100,0	43,22	30,70	34,98	**57,06**

1/32 1/16 1/8 1/4 1/2 1 2 4 8 16 32 -fold mean IC_{50}

sensitive cell lines — resistant cell lines

Figure 66. Heatmap presentation of individual IC_{50} values of eight compounds (**39**, **43-47**) in a panel of 12 human tumor cell lines.

For a deeper exploration of tumor selectivity, compound **47** was tested in a panel of 42 cell lines covering 15 different tumor histotypes. Across this broad panel, a mean IC_{50} value

of 5.858 µM was detected, with pronounced activity towards the renal cancer cell line RXF 393 (IC_{50} = 1.143 µM). In addition, the cell lines HT-29 (colon), MEXF 1341 (melanoma) and OVXF 899 (ovary) showed above-average sensitivity.

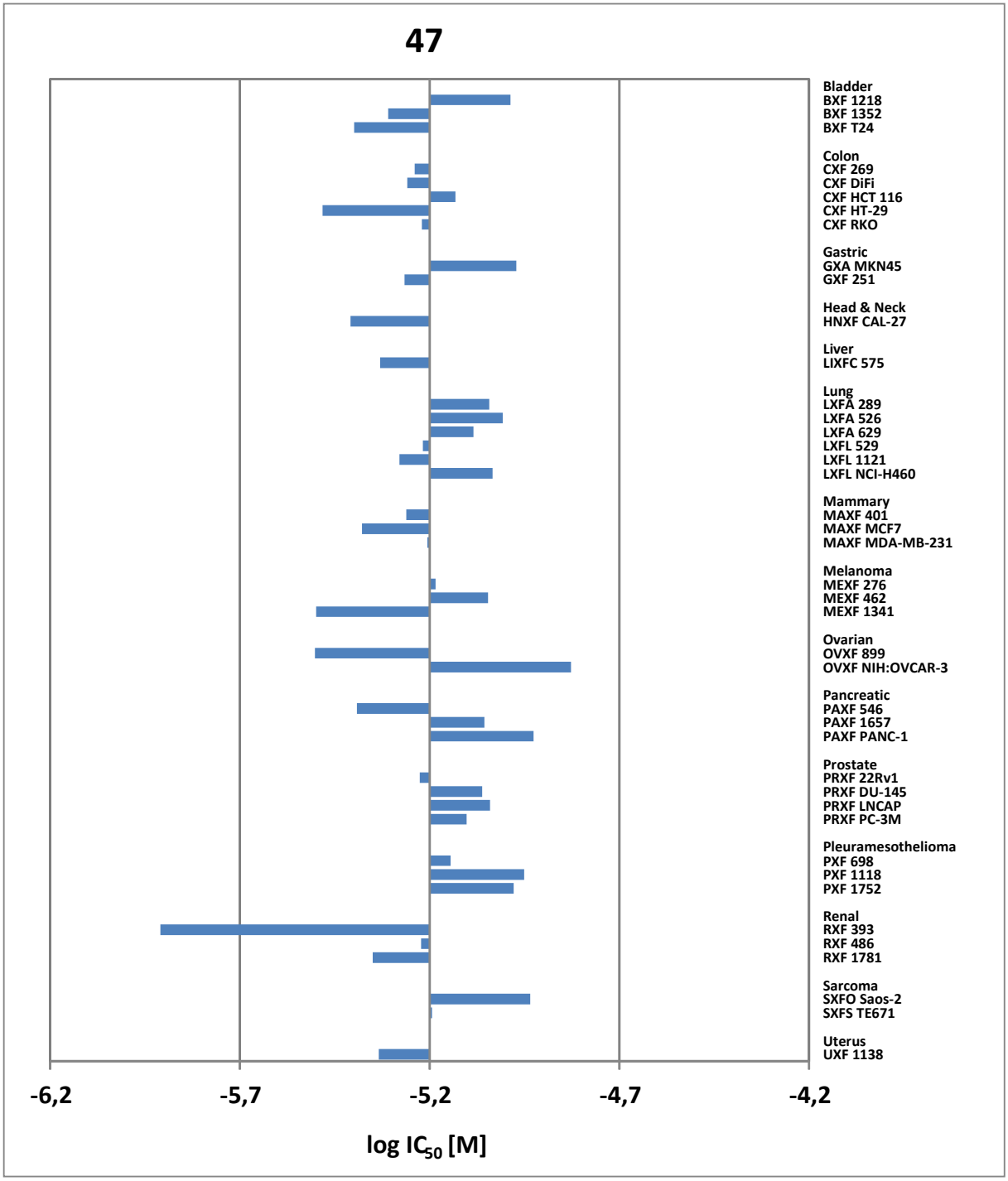
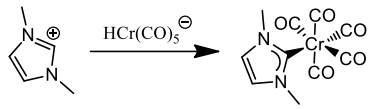


Figure 67. IC_{50} mean graph presentation for compound **47** in a panel of 42 human tumor cell lines.

IV. Results and discussion. N-Heterocyclic Carbenes (NHC) derivatives containing 1,2,4-oxadiazole motif

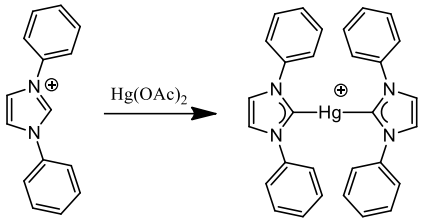
IV.1. General Introduction

The starting point of N-heterocyclic carbenes (NHC) chemistry and the related transition metal complexes began in 1968, when Öfele[238] reported the first transition metal NHC complex by deprotonating an imidazolium salt with a metal hydride (Scheme 43).



Scheme 43. Pioneer transition metal NHC complex reported by Öfele.

At the same time, as a result of parallel work, Wanzlick and Schönherr[239] published independently another metal NHC complex obtained by treating the imidazolium salt with the acetate salt of the metal (Scheme 44).



Scheme 44. Pioneer transition metal NHC complex reported by Wanzlick.

Several years later, in 1975, the first functionalized NHC metal complexes reported by Clarke and Taube[240] entered the field of biochemistry. They coordinated ruthenium to xanthine derivatives through the carbenic carbon of the imidazole.

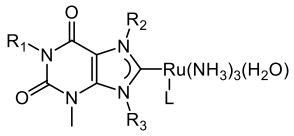
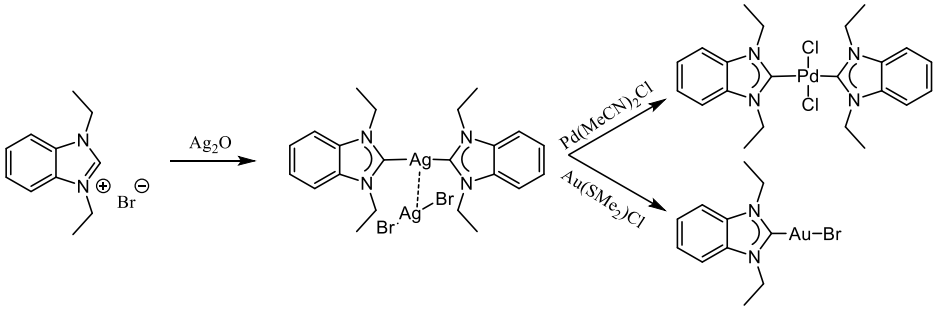


Figure 68. Xanthine NHC complexes of Ruthenium.

The isolation of the first free stable carbene by Arduengo *et al.*[241] in 1991 represented a breakthrough in the chemistry of transition metal carbene complexes. In the next two and a half decades the chemistry of NHC compounds and their metal complexes,[242-246] along with catalysis application[247] flourished. Even if the NHC chemistry has flourished in the last 25 years, their practical use has been limited by the instability of the free carbenes. Nevertheless, the imidazolium salt precursors can be handled with less difficulty and they represented the

ground for another significant step in the development of NHC chemistry. In 1998 Lin and Wang developed a reaction procedure in which the silver(I)-NHC complex was generated from the treatment of an imidazolium salt with Ag_2O (Scheme 45).[248] This silver(I)-NHC compound can then serve as a carbene transfer agent for transition metals.[249,250]

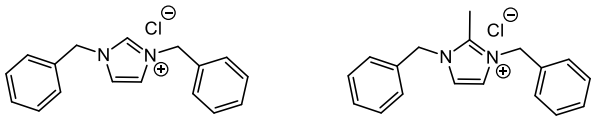


Scheme 45. Silver(I) intermediate route reported by Lin and Wang.

IV.2. Synthesis and structural characterization of imidazolium salts

IV.2.1. General Aspects

Imidazolium salts are imidazole derivatives in which the two nitrogen atoms of the heterocycle are alkylated. Furthermore they are biologically active, and particularly known for their antitumor properties.[251-254] However, at the same time their natural abundance is scarce. Figure 69 depitcs two natural compounds isolated from *Lepidium meyenii*, Lepidiline A and Lepidiline B, which manifest strong cytotoxicity against tumor cell lines.[255]



Lepidiline A Lepidiline B

Figure 69. Natural imidazolium salts derivatives.

Besides the medicinal aspect, the imidazolium salts may also act as ionic liquids at ambient temperature [189,256] and as the main precursors for N-heterocyclic carbenes (NHCs) or bis-imidazolidines, which are known for their application in the synthesis of organic compounds.[257-259]

IV.2.2. Synthetic aspects

Numerous preparative protocols are described in the literature for the synthesis of imidazolium salts.[196] The three most frequently employed methods are represented in Figure 70. One pathway involves the formation of a Schiff base intermediate as a coupling result

between the amine and glyoxal under acidic conditions (Path A). The diimine can be isolated or can be reacted further with formaldehyde generating the corresponding imidazolium salt.[260-262] This method has its limitations in the sense that just primary amines can be used as starting materials and only symmetrically substituted imidazolium salts can be produced.

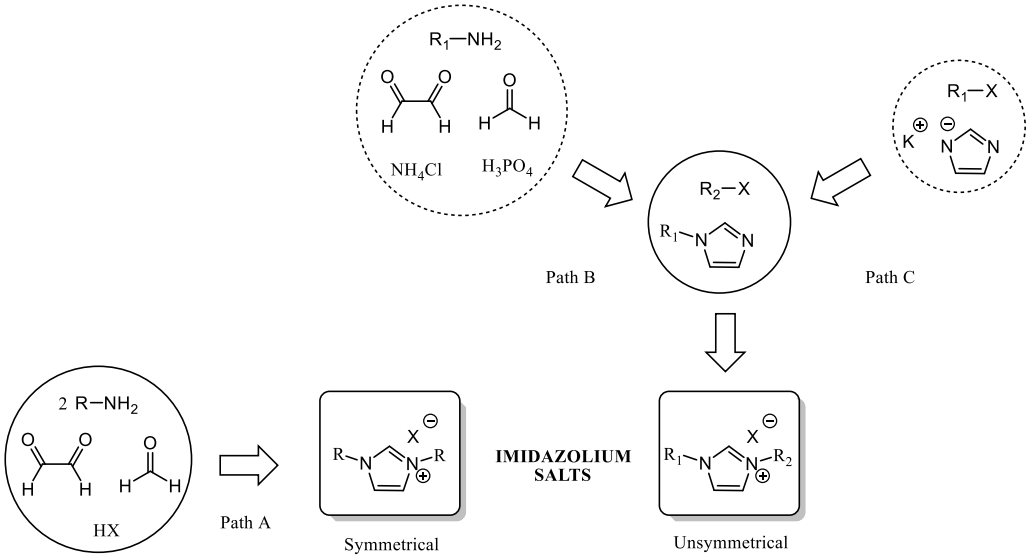
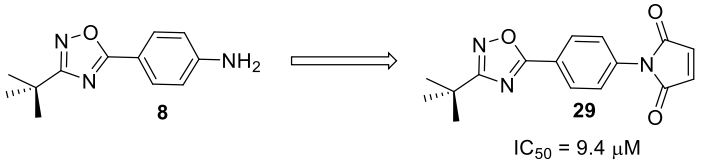


Figure 70. General representation for the synthesis of imidazolium salts.

The other two routes for the synthesis of imidazolium salts (usually unsymmetrically) required the formation of an *N*-substituted imidazole derivative. This key intermediate can be generated using a one-pot reaction among glyoxal, ammonium chloride, paraformaldehyde and amine (Path B), or by alkylating the potassium imidazolide (Path C). After the *N*-substituted imidazole is generated, another alkylation with an appropriate alkyl, acyl or benzyl reagent will provide the corresponding imidazolium salts.[263,264]

IV.2.3. Motivation

In previous work, it was described the synthesis of several natural product analogues starting from 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)aniline (**3**).[231] All derivatives were tested *in vitro* for antitumor activity towards a panel of 11 cell lines by using a monolayer cell survival and proliferation assay.



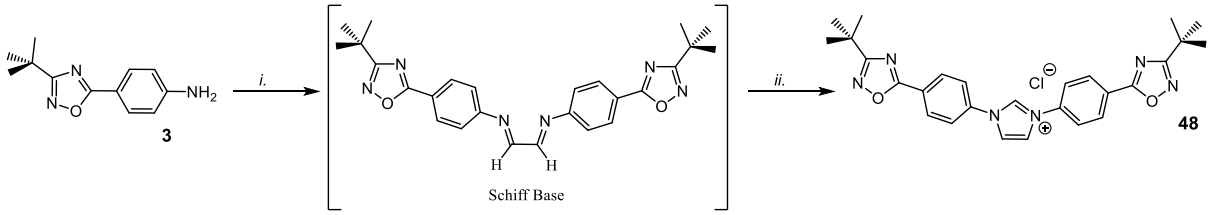
Scheme 46. Reported 1,2,4-oxadiazole bearing maleimide moiety.

One of the compounds, 1-(4-(5-(*tert*-butyl)-1,2,4-oxadiazol-3-yl)phenyl)-1*H*-pyrrole-2,5-dione (**29**) (Scheeme 46), showed good anti-tumor potency combined with good selectivity, with an IC_{50} mean value of 9.4 μM. Taking these results into consideration, it was envisioned to replace the maleimido group by an imidazole core. This exchange opens the road to the chemistry of 1,2,4-oxadiazole-containing NHC-metal complexes, which could lead to an improved antitumor activity in the target molecules. Starting from 5-(4-(1*H*-imidazol-1-yl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (**8**), the idea beyond was to generate the imidazolium salts that incorporate 1,2,4-oxadiazole unit, which would be interesting precursors for NHCs. The imidazolium salts can be created with particular hydrophobic substituents attached to the imidazole heterocycle. With these compounds containing the 4-(5-(*tert*-butyl)-1,2,4-oxadiazol-3-yl)phenyl group in hand, I intended to synthesize organometallic complexes.

IV.2.4. Synthesis of symmetrical imidazolium salts containing 1,2,4-oxadiazoles.

IV.2.4.1 Synthesis of 1,3-bis(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-imidazolium chloride (48)

The symmetrical imidazolium chloride **48** was generated directly from the amine **3** using literature-reported protocols [265,266] in a one-pot reaction as shown in Scheme 47. The practical work was straightforward. The primary amine **3** was refluxed in toluene along with aqueous glyoxal, paraformaldehyde and HCl. After removing the volatiles and several trituration procedures with acetone, the desired 1,2,4-oxadiazole-containing imidazolium chloride was obtained in satisfactory yield (55%).



Scheme 47. Synthesis of (1,3-bis(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)imidazolium chloride (**48**) *i.* glyoxal, toluene; *ii.* paraformaldehyde, HCl, toluene, reflux.

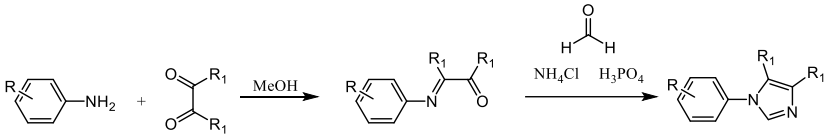
Attempts to isolate the Schiff base intermediate as pure compound for a proper chemical characterization were unsuccessfully, mainly caused decomposition during the purification procedure. The chemical structure of the final imidazolium salt was confirmed by ^{1}H NMR spectroscopy and MS (HR-ESI) spectrometry. The characteristic signal for the N-*CH*-N proton was registered at 10.76 ppm. Due to the low solubility of the compound the ^{13}C NMR

spectra could not be measured. The HR-ESI also confirmed the charged mass signal of the molecule.

IV.2.5. Synthesis of unsymmetrical imidazolium salts containing 1,2,4-oxadiazoles.

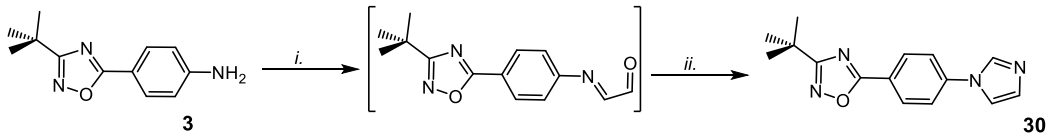
By constructing the unsymmetrical imidazolium salts, it could be systematically inserted besides the 1,2,4-oxadiazole unit another second substituent. As second substituents were chosen some commonly used units (methyl, benzyl) and a wide variety of other organic moieties that are known to be biologically active, *i.e.* anthracene, indol, 2-pyridine, 2,3,4,5-tetra-*O*-acetyl-*D*-glucopyranose, quincorine (QCI) and quincoridine (QCD). These groups should influence the transport of the final agents to the target cells.

The unsymmetrical imidazolium salts were synthesized following a two steps protocol starting from compound **3**. In the first step, the *N*-substituted imidazole **30** was generated using classic literature-reported protocol reported by Liu *et al.* (Scheme 48).[200] He developed a two-step protocol for the synthesis of 1-arylimidazoles in good yields using different substituted aniline compounds.



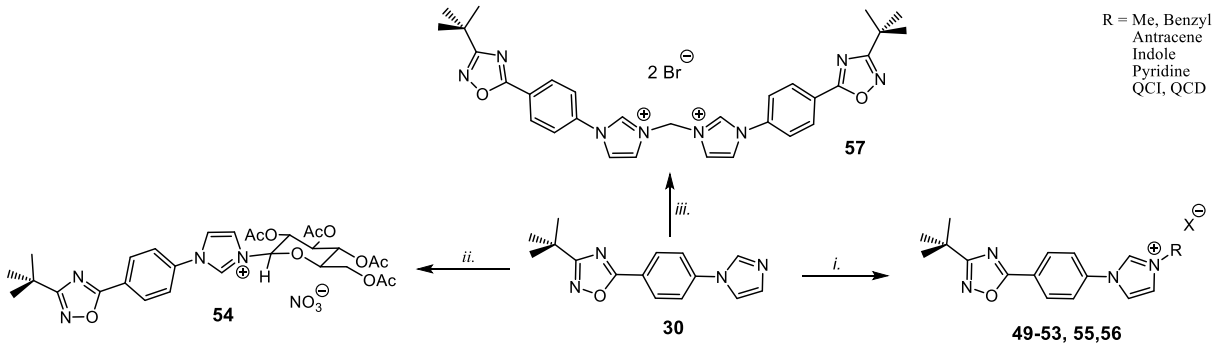
Scheme 48. Synthesis of 1-arylimidazoles reported by Liu *et al.*.

First, a phenyl-imino-ketone intermediate was developed *in-situ* by reacting the amine with glyoxal derivatives in methanol at room temperature. Then, the imidazole ring was closed in the presence of H_3PO_4, NH_4Cl and formaldehyde at reflux. Using this method, the 1,2,4-oxadiazole amine xx was treated with glyoxal and then with formaldehyde, NH_4Cl and H_3PO_4. The final imidazole substituted with 1,2,4-oxadiazole unit was obtained in acceptable yields (48-50%).



Scheme 49. Synthesis of imidazole derivative starting from 4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)aniline (**8**); *i.* glyoxal, MeOH, rt; *ii.* NH_4Cl, 37% aq. formaldehyde, H_3PO_4, MeOH, reflux.

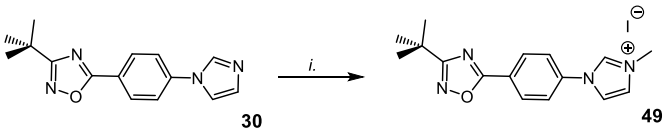
After having prepareda stock solution of 5-(4-(1H-imidazol-1-*yl*)phenyl)-3-*tert*-butyl-1,2,4-oxadiazole nine imidazolium salts **49**-**57** with various counter ions (Cl^-, Br^-,I^- or NO_3^-) were generated were generated from the alkylation methodology (Scheme 50).



Scheme 50. General synthesis of 4-(3-(*tert*-butyl)-1,2,4-oxadiazol imidazolium salts; *i.* R-X (Cl, Br, I) toluene, reflux; *ii.* 2,3,4,5-tetra-*O*-acetyl-*D*-glucopyranosylbromide, $AgNO_3$, CH_3CN, 50°C; *iii.* CH_2Br_2, reflux.

IV.2.5.1. Synthesis of 1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-methyl-imidazolium iodide (49)

The synthesis of the 1,2,4-oxadiazole-containing imidazolium salts started with 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-methyl-imidazolium iodide (**49**), the most straightforward from all imidazolium salts. The alkylation with MeI in THF was fast, clean and proceeded with excellent yield (98%).

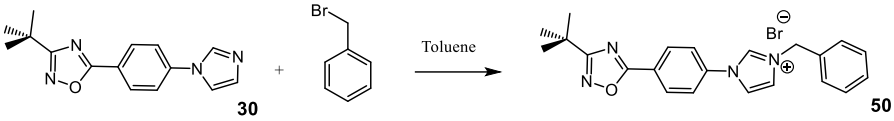


Scheme 51. Synthesis of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-methyl-imidazolium iodide (**49**); *i.* MeI, THF, rt.

The new compound was characterized by IR, ^1H NMR, ^{13}C NMR spectroscopy and MS (HR-ESI). The characteristic N-*CH*-N resonances were observed in the ^1H and ^{13}C NMR spectra at 9.98 and 137.4 ppm, respectively. The HR-ESI revealed, as expected, the charged masse signal of the molecule, without the iodine counterion.

IV.2.5.2. Synthesis of 1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(benzyl)-imidazolium bromide (50)

The next alkylating agent used in the synthesis of novel 1,2,4-oxadiazole-containing imidazolium salt was the inexpensive and commercially available benzyl bromide.

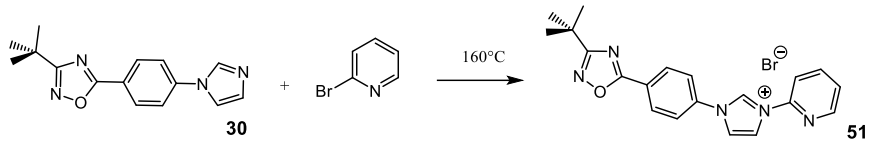


Scheme 52. Synthesis of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(benzyl)-imidazolium bromide (**50**).

This reaction proceeded as expected. The two reagents were mixed equimolar quantities in toluene under reflux and the solid obtained after precipitation with diethylether was properly characterized. In the ^{1}H NMR spectrum, the NHC proton at 10.41 ppm and the two C*H*$_2$-bridge protons at 5.61 ppm, both display as singlets. The HR-ESI result is also in good correlation with the expected charged mass.

IV.2.5.3. Synthesis of 1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2-pyridine)-imidazolium bromide (51).

Another alkylating agent used intensively in the design of imidazolium salts and their corresponding NHC carbenes is the 2-bromopyridine molecule.[267-269] Usually this unit is inserted in the NHC chemistry in order to obtain pincer type complexes with catalytic applications.



Scheme 53. Synthesis of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2-pyridine)-imidazolium bromide (**51**).

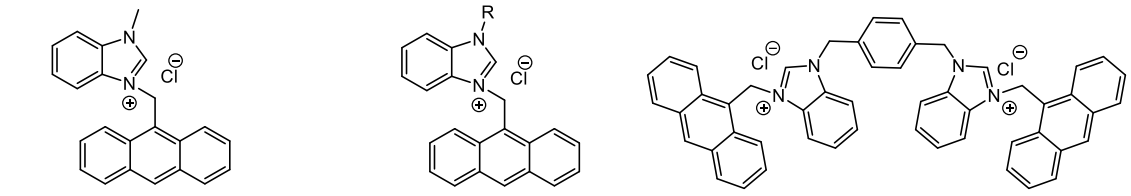
The imidazolium salt **51** was generated after reacting the 1,2,4-oxadiazole-imidazole with an excess of 2-bromopyridine for 48 hours at elevate temperature (160°C). The resulting salt containing the pyridine ring as second substituent was obtained in excellent yield and purity. The structure of the new compound correlates to the registered analytics. Besides the signals attributed to the phenyl, backbone imidazole and pyridine rings, the ^{1}H NMR spectra shows clearly the C_2 proton of the imidazolium ring at 10.88 ppm.

Until this point all synthetic work related to the generation and isolation of the imidazolium salts (**48**-**51**) in good yields and purity turned out to be straightforward. The implementation of more sterically demanding and sensible groups on the imidazole ring turns out to be quite challenging from a synthetic point of view. Starting with the introduction of 9-methyl anthracene things started becoming more complicated.

IV.2.5.4. Synthesis of 1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(9-methyl-anthracene)-imidazolium chloride (52).

The number of reported imidazolium salts that contains the anthracene skeleton is rather small. Most of the papers investigate the fluorescent properties of these derivatives along with the cation-anion interaction in imidazolium salts.[270,271] The inspiration for the addition of the fluorescent antracenyl ligand on the 1,2,4-oxadiazole-imidazole core came

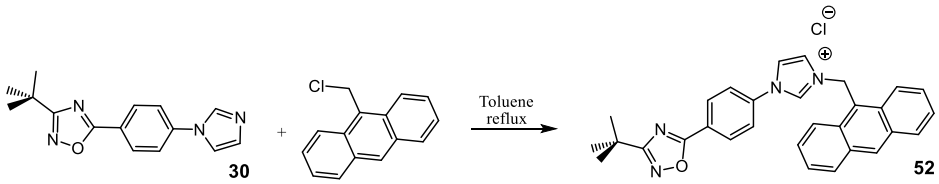
from the work of Citta *et al.*[272] who reported the synthesis of an antracenyl imidazolium salt used as precursor for two NHC complexes which were later screened for their cytotoxic properties against human ovarian cell lines resistant to cisplatin and for nontumorigenic human kidney cell lines. Karatas *et al.* also investigated the activity of several antracenyl-containing benzimidazole derivatives as tyrosinase inhibitors.[273]



Imidazolium salts reported by Citta et al. Imidazolium salts reported by Karatas et al.

Figure 71. Reported imidazolium salts which include the antracenyl motif.

In order to obtain the oxadiazole-antracenyl-imidazolium salt **52**, the imidazole derivative **30** had to be refluxed in toluene for several days (Scheme 54). Although the yields for other antracenyl imidazolium salts were reported as good to very good (50-90%), in my case the yield of pure substance did not exceed 24%. One reason for this is that the reaction was never complete and the only purification method available was the product precipitation from toluene.



30 **52**

Scheme 54. Synthesis of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(9-methyl-anthracene)-imidazolium chloride (**52**).

The new synthesized compound was characterized by IR, ^{1}H NMR and ^{13}C NMR spectroscopic methods and by mass spectrometry (HR-ESI). In the ^{1}H NMR spectra it was possible to observe the particular N-*CH*-N resonance at 10.49 ppm as singlet along with the protons belonging to the anthracene skeleton and 1,2,4-oxadiazole ligand. The ^{13}C NMR spectrum was also in accordance with the structure of the novel compound. The N-*C*-N signal was registered at 147.2 ppm. Furthermore, the HR-ESI result was congruent with the charged molecule.

IV.2.5.5. Synthesis of 1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-((2-ethyl)indole)-imidazolium bromide (53).

3-Substituted indole is another unit worth incorporating into the 1,2,4-oxadiazole containing imidazolium salts. This moiety is present in several natural products known for their broad spectrum of bioactive properties, e.g. antitumor activity.[274,275] Figure 72 displays two representative natural compounds containing 3-substituted indole, Trachycladindole E and Hyrtinadine A.

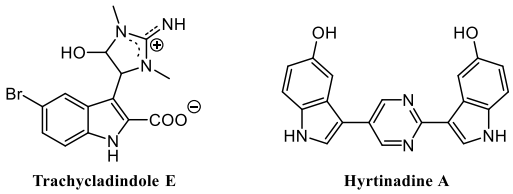
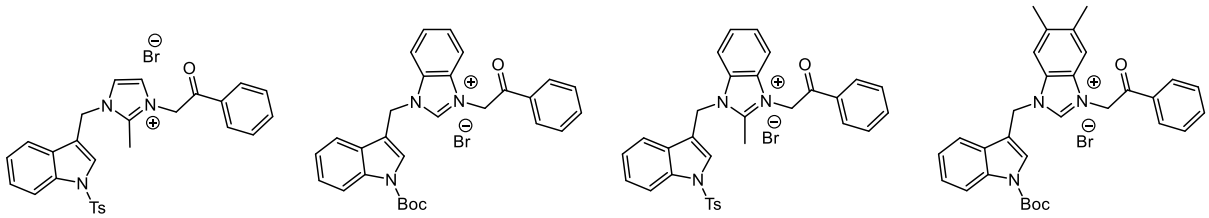


Figure 72. Structures of natural products that incorporate the 3-substituted indole unit.

The first one belongs to a group of cytotoxic agents Trachycladindoles A-G extracted from the Australian marine sponge *Trachycladus laevispirulifer*. This class of substances show selective cytotoxicity against lung (A549), colorectal (HT29) and breast (MDA-MB-231) cancer cell lines.[276] Hyrtinadine A is a cytotoxic bis-indole alkaloid isolated from a marine sponge, *Hyrtios* sp..[277] Although the indole-containing compounds have remarkable biological activities, this moiety has so far not been considered as a fragment for the construction of imidazolium salts or related NHC derivatives. The only literature example is represented by the work of X.-L. Xu *et al.*.[278] They synthesized a series of new 1-((indol-3-yl)methyl)-1*H*-imidazolium salts and tested them *in vitro* against several human cell lines.

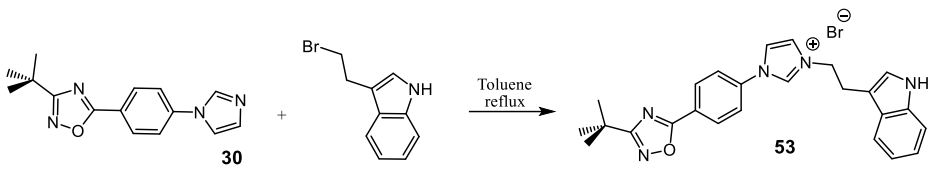


Fgure 73. Reported 1-((indol-3-yl)methyl)-1*H*-imidazolium salts.

Some of these compounds turned to be good candidates against myeloid liver carcinoma (SMMC-7721), lung carcinoma (A549), and breast carcinoma (MCF-7).

In order to integrate the cytotoxic activity of 3-substituted indole into the 1,2,4-oxadiazole-imidazole, the commercially available 3-(2-bromoethyl)indole was used as alkylating reagent. The ethyl arm of the indole was chosen because of its availability, cost and advantageous steric properties. The yield of this reaction was satisfactory (20%) considering

the sensitivity of the reagent involved and the fact that many flash chromatography purifications were required.



Scheme 55. Synthesis of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-((2-ethyl)indole)-imidazolium bromide (**53**).

The pure compound was characterized IR, ^{1}H NMR and ^{13}C NMR spectroscopy. Characteristic for this compound are the ^{1}H NMR singlet resonances at 11.04 ppm and 10.09 ppm belonging to the NH_{Indole} and the NC*H*N, respectively. The ethyl bridge gave signals at 4.6 ppm and 3.43 ppm. The calculated HR-ESI mass for M^{+} (412.2137) agrees with the experimentally determined one (412.2135).

IV.2.5.6. Synthesis of 1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2,3,4,5-tetra-O-acetyl-D-glucopyranosyl)-imidazolium nitrate (54).

Ubiquitous carbohydrates represent a special class of *N*-alkyl ligands for NHCs that have so far been neglected for the biological applications. They play a crucial biological role in the complex mechanisms of living organisms (storage and transport of energy, key function in the immune system, fertility, pathogenesis, blood clotting).[279-281] Hence, carbohydrates are ubiquitous building blocks in the design of novel bioactive compounds.[282]

As substituents they have the advantage of their intrinsic chirality and water solubility. Nevertheless, carbohydrate-based NHCs and their imidazolium precursors are an emerging field and so far their applications are confined to catalysis,[283-288] but not as possible bioactive agents. Figure 74 shows the carbohydrate-based imidazolium salts so far reported in the literature.

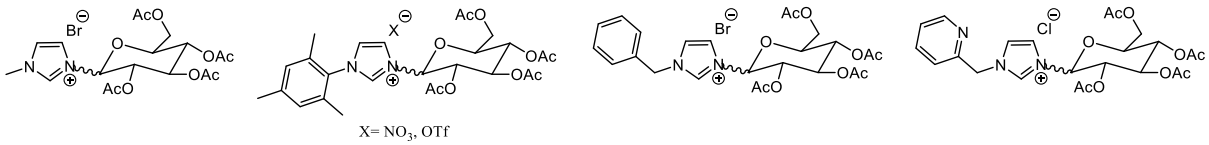
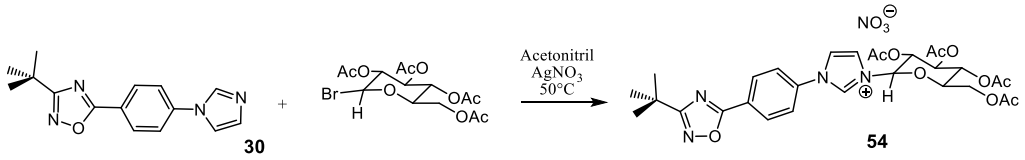


Figure 74. Reported carbohydrate-based imidazolium salts in catalytic studies.

Therefore with the purpose of establishing a new structural class of highly active, stable, stereo-selective bioactive compounds, the synthesis of 1,2,4-oxadiazole-carbohydrate-based imidazolium salt and their NHCs derivatives was attempted.



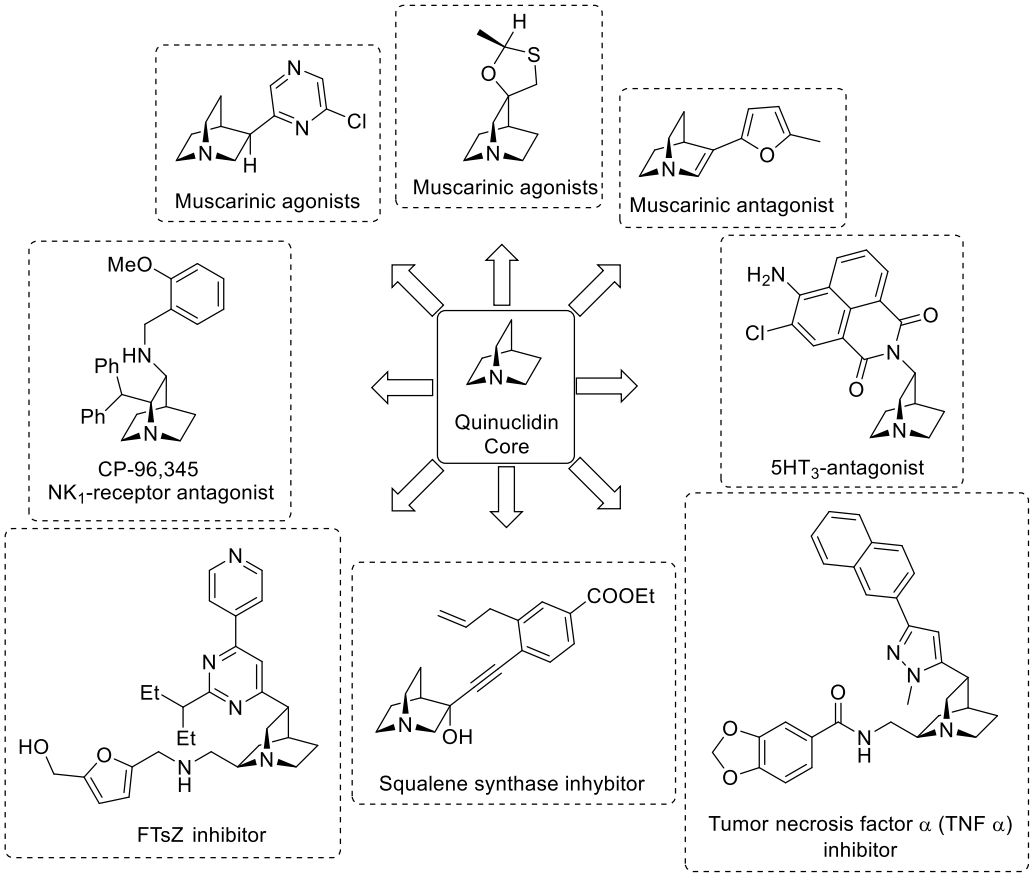
Scheme 56. Synthesis of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2,3,4,5-tetra-O-acetyl-D-glucopyranosyl)-imidazolium nitrate (**54**).

As showed in Scheme 56, the 1,2,4-oxadiazole substituted imidazole derivative **30** was treated with a suitable acetyl protected glucose bearing a bromine atom at the anomeric site as leaving group. The reaction was performed at 50°C in the presence of $AgNO_3$ as bromide abstractor. After work-up the remaining residue was a anomeric mixture of isomers from 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2,3,4,5-tetra-*O*-acetyl-D-glucopyra-nosyl)-imidazolium nitrate. It is worth mentioning that despite the low yield (18%) it was obtained solely the *β*-anomer by combining precipitation from methanol and chromatography methods.

The ^{1}H NMR spectroscopy revealed the *β*-configuration of the new carbohydrate imidazolium salt. The proton attributed to the anomeric center gives in the ^{1}H NMR spectra a doublet at 6.46 ppm with the coupling constant J_{HH} = 8.6 Hz. The other ^{1}H NMR specific signals attributed to the carbohydrate moiety along with the imidazolium C_2 proton signal confirm the structure of the new compound.

IV.2.5.7. Synthesis of 1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCI)-imidazolium bromide (55) and 1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCD)-imidazolium bromide (56).

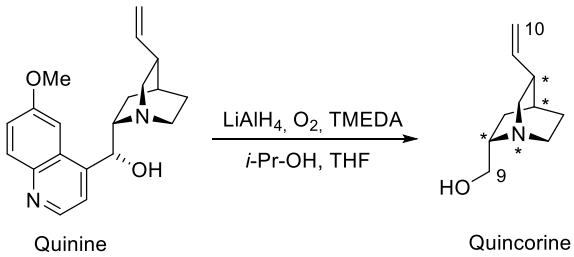
Quinuclidine containing compounds have a variety of pharmacological properties. In Scheme 57 the most relevant derivatives possessing the quinuclidinic core from the medicinal point of view are summarized. Some of them are very strong muscarinic agonists and antagonists,[34,289] others like CP-96345 are NK_1-receptor antagonist,[290-292] 5-HT_3-antagonists[293,294] and Squalene synthase inhibitors.[295,296] More recent studies reported that quinuclidine-containing compounds are inhibitors agents against Tumor Necrosis Factor α (TNF α)[297] and can act as antimicrobial FTsZ (Filamenting temperature-sensitive mutant Z) inhibitors.[298]



Scheme 57. Examples of quinuclidine containing compounds with medicinal aplications.

One available source of this bioactive core is the family of *cinchona* alkaloids and their derivatives, well-known for their application in medicine,[299] food industry [source of bitterness] and asymmetric catalysis.[300-302]

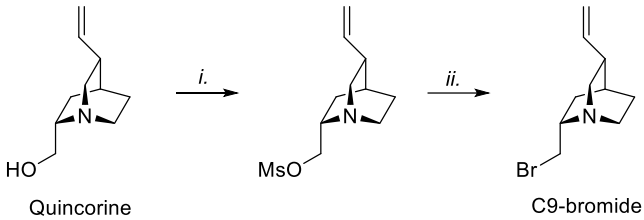
The two members used as quinuclidinic nucleus in the design of new chiral imidazolium salts are quincorine [QCI = (2*S*,4*S*,5*R*)-2-hydroxymethyl-5-vinyl-2-quinuclidine] and quincoridine [QCD = (2*R*,4*S*,5*R*)-2-hydroxymethyl- 5-vinyl-2-quinuclidine]. These two pseudo-enantiomeric 1,2-amino alcohols were obtained by Hoffmann *et al.*[303] as a cleavage result of the natural products quinine and quinidine.



Scheme 58. Cleavage of quinine into QCI.

The four stereogenic centers along with the possibility of functionalization at both ends (C9 and C10) make the QCI and QCD skeletons attractive building blocks in the synthesis of novel compounds.[304-310]

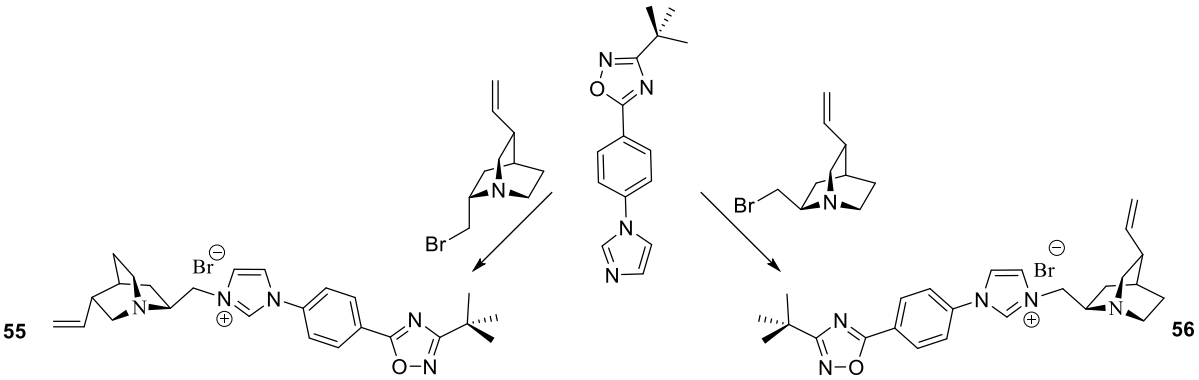
The synthetic strategy was quite simple: Use a quinuclidine derivative as alkylating agent and implement it on the imidazole already substituted with 1,2,4-oxadiazole unit. However, the practical synthesis proved to be more complicated. The first step was the synthesis of the C9-halogenated quincorine and quincoridine derivatives. Following the work of Hoffmann *et al.*[311] it was possible to obtain the C9-bromide derivative of QCI and QCD, respectively (Scheme 59).



Scheme 59. Two step synthesis of C9-bromide derivative of QCI: *i.* Et3N, MsCl, DCM, rt; *ii.* LiBr, dioxane, reflux.

The unprotected quincorine and quincoridine were first transformed into the corresponding *O*-mesylated compounds. After isolation and purification, these derivatives were reacted with LiBr in refluxing dioxane for several hours to afford after column chromatography the desired C9-bromides in good yields, which is remarkable considering that these compounds are very sensitive.

After the isolation of these brominated compounds, the next step was the alkylation reaction with 5-(4-(1H-imidazol-1-*yl*)phenyl)-3-*tert*-butyl-1,2,4-oxadiazole (**30**) at 120°C in toluene.



Scheme 60. Alkylation of 1,2,4-oxadiazole imidazole with the C9-bromide QCI/QCD.

The isolated yields were not consistent from run to run. Sometimes the imidazolium salts were obtained in 15% yield, sometimes in 65% yield. The main reason for this is that

QCI- / QCD-C9-Br tend to react with themselves at elevated temperatures to form bromide salts, thus becoming inactive as electrophiles (Figure 75). This problem was solved by adding more equivalents of the QCI- / QCD-C9-Br and by heating for shorter period of time.

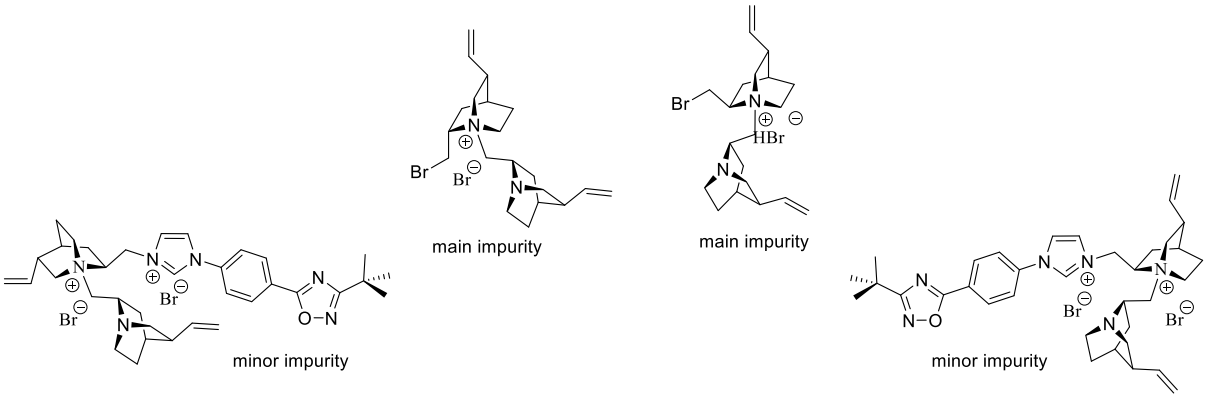


Figure 75. Self-alkylation of C9-bromide QCI/QCD identified in the MS (ESI).

The formation of the two chiral imidazolium salts 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCI)-imidazolium bromide (**55**) and 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCD)-imidazolium bromide (**56**) was confirmed by IR, ^{1}H and ^{13}C NMR spectroscopy. Apart from the complex signals of the quinuclidinic core, in the ^{1}H NMR spectrum the characteristic signals for the carbenic proton (QCI-9.26 and QCD-10.96 ppm), the backbone of the imidazole ring and the rest from the 1,2,4-oxadiazole part (*tert*-butyl and phenyl) can be found. Even more, the two imidazolium salts were unambiguously confirmed by HR-ESI mass spectrometry.

IV.2.5.8. Synthesis of 1,1′-[4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl]-3,3′-methylenediimidazolium bis-(bromide) (57).

The synthesis of bis-imidazolium salt containing 1,2,4-oxadiazole unit was mainly inspired by the work of Haque *et al.*.[312,313] They reported the synthesis and the antitumor activity of some bulky bis-imidazolium salts with octyl, nonyl and decyl terminal chains (Figure 76).

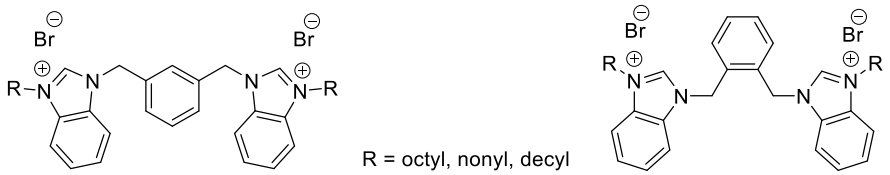
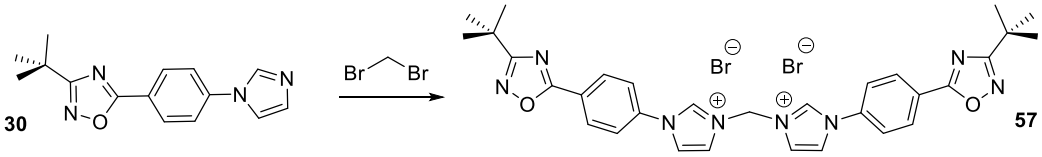


Figure 76. Structures of reported bis-imidazolium salts with antitumor activity.

The compounds were investigated for their cytotoxic abilities against HCT-116 (human colon cancer), HT-29 (human colorectal adenocarcinoma) and MCF-7 (human breast

adenocarcinoma) cell lines. Moreover, they showed anti-proliferative selectivity against HT-29 cell line.

The synthesis of 1,1′-[4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl]-3,3′-methylene-diimidazolium bis-(bromide) (**57**) started from the 5-(4-(1H-imidazol-1-yl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole and dibromethane. The reaction was straightforward and formed the product in good purity and yield (78%)



Scheme 61. Synthesis of 1,1′-[4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl]-3,3′-methylene-diimidazolium bis-(bromide) (**57**).

The new bis-imidazolium salt was characterized by IR, ^{1}H NMR spectroscopy and mass spectrometry (HR-ESI). At 10.56 ppm in the ^{1}H NMR spectra is visible the typical NC*H*N singlet signal integrated for two protons. Another notable signal is the methylene bridge at 7.02 ppm as a sharp singlet. The presence of the two imidazolium rings is also confirmed by the HR-ESI mass spectrometry which shows half of the double charged molecular mass.

IV.2.5.9. Remarkable aspects in ^{1}H and ^{13}C NMR spectroscopy of the 1,2,4-oxadiazole related imidazolium salts.

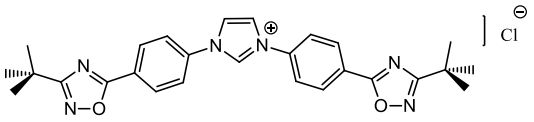
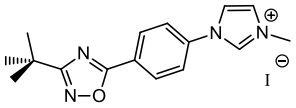
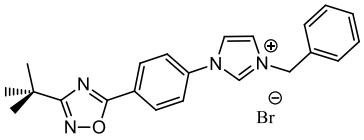
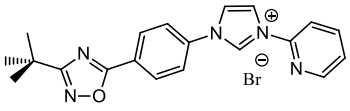
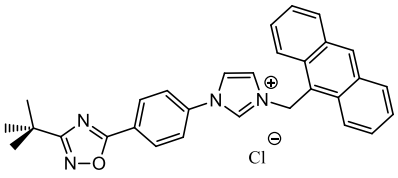
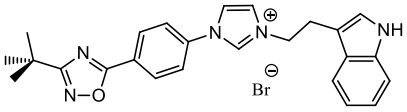
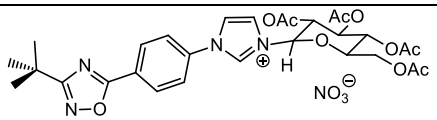
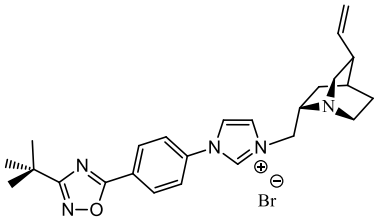
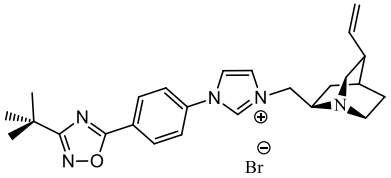
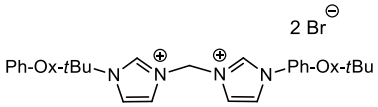
Imidazolium salt	Solvent	δ_H *[ppm]* (NHC*HX)	δ_C*[ppm]* (NHC*HX)
48 	DMSO-d6	10.76	b)
49 	DMSO-d6	9.98	137.4
50 	DMSO-d6	10.41	137.8
51 	DMSO-d6	10.88	137.6
52 	DMSO-d6	10.49	147.2
53 	DMSO-d6	10.09	138.4
54 	$CDCl_3$	11.09	137.8
55 	MeOH-d4	9.26	139.3
56 	$CDCl_3$	10.96	136.6
57 	DMSO-d6	10.56	b)

Table 4. Notable features in the ^{1}H and ^{13}C NMR spectra of the imidazolium salts.

In Table 4 are summarize notable features in the ^{1}H and ^{13}C NMR spectra of the imidazolium salts. The imidazolium protons resonante between 9.26 and 11.09 ppm, whereas the corresponding imidazolium carbons can be found at ca. 136.57-147.23 ppm.

IV.2.6. *In vitro* anti-tumor activity of 1,2,4-oxadiazole-containing imidazolium salts towards human tumor cell lines.

The imidazolium salts **48**-**57** were tested compare the anti-tumor activity after the incorporation of a metal. Furthermore, in recent publications [313,314] the anti-tumor activity of imidazolium salts was described, so there is a general interest in their activity.

The *in vitro* anti-tumor activity of the imidazolium salts compounds **48**-**57** was assessed in a panel of 12 human tumor cell lines by using a monolayer cell survival and proliferation assay. As presented in Figure 77, good potency with mean IC_{50} values <10 µM was detected for one compound, namely **49**. In addition compound **52** presented a moderate activity (mean IC_{50} = 25.4 µM). The other tested compounds (**48**, **50**, **51**, **53**-**57**) showed marginal or none *in vitro* anticancer activity.

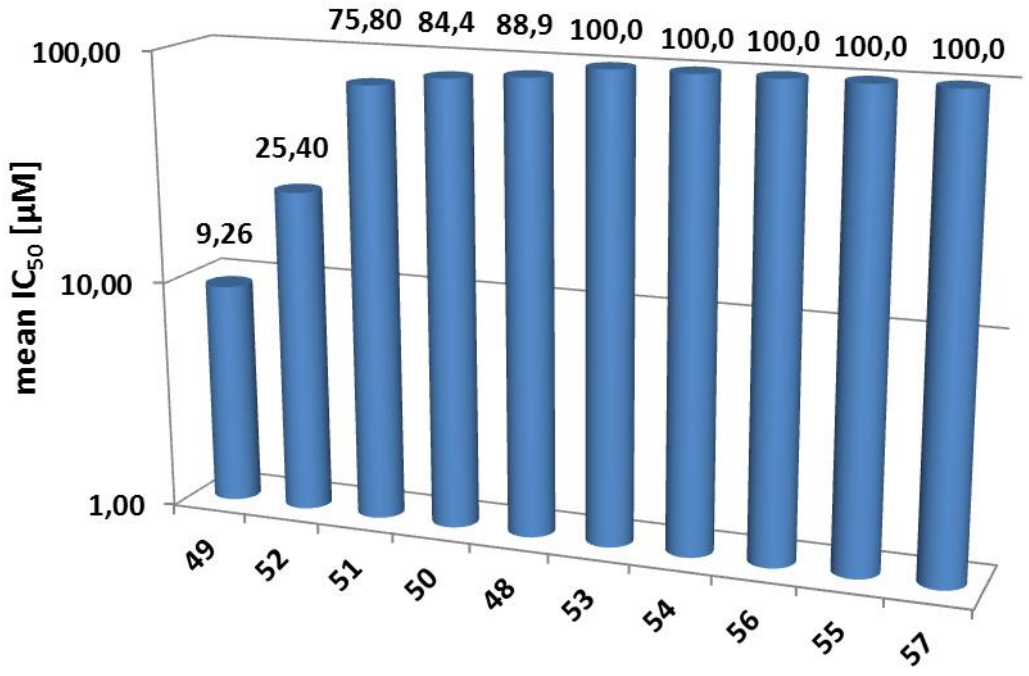
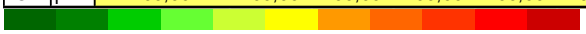


Figure 77. In vitro anti-tumor activity of compounds **48**-**57** in a panel of 12 human tumor cell lines (mean IC_{50} values).

Although is an accepted fact that after the incorporation of metal to an imidazolium salt through metal-NHC bond enhances its activity, compound **49** has an activity higher than the anti-tumor activity of the corresponding Au(I) complex. Compound **49** is in some aspects

different from the remaining imidazolium salts. It features the smallest substituent (a methyl-group), possesses an iodide counterion and it also has the best solubility in organic solvents.

		Cell lines												
Compound	Unit	CXF HT-29	GXF 251	LXFA 629	LXFL 529	MAXF 401	MEXF 462	OVXF 899	PAXF 1657	PRXF 22Rv1	PXF 1752	RXF 486	UXF 1138	geomean IC50 [µM]
49	µM	13,88	2,12	6,47	9,63	5,68	9,56	44,76	7,13	5,18	22,27	22,58	4,79	**9,26**
52	µM	39,39	8,99	24,60	16,46	20,51	26,99	100,00	28,40	10,66	39,48	100,00	7,60	**25,40**
51	µM	100,00	29,28	62,87	89,83	100,00	100,00	100,00	100,00	44,36	100,00	100,00	49,00	**75,79**
50	µM	100,00	94,12	100,00	37,89	100,00	92,32	100,00	100,00	48,30	100,00	100,00	81,82	**84,37**
48	µM	100,00	24,53	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	**88,95**
53	µM	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	**100,00**
54	µM	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	**100,00**
56	µM	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	**100,00**
54	µM	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	**100,00**
57	µM	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	**100,00**



1/32 1/16 1/8 1/4 1/2 1 2 4 8 16 32 -fold mean IC_{50}

sensitive cell lines resistant cell lines

Figure 78. Heatmap presentation of individual IC_{50} values for compounds **3-12** in a panel of 12 human tumor cell lines.

The individual IC_{50} values of the tested compounds are presented in Figure 78. Compound **49**, the most active from the imidazolium salts showed an individual IC_{50} values in the area 2.12 µM (GXF 251) to 44.76 µM (OVXF 899), corresponding to a 21-fold difference between the most sensitive and the least sensitive cell line. This shows a high level of tumor selectivity. Interestingly, four of the tested compounds (**49**, **52**, **51** and **48**) presented in their activity profile selectivity pattern against the gastric tumor cell line (GXF 251).

IV.3. Synthesis and structural characterization of gold(I)-NHC complexes with 1,2,4-oxadiazole substituents

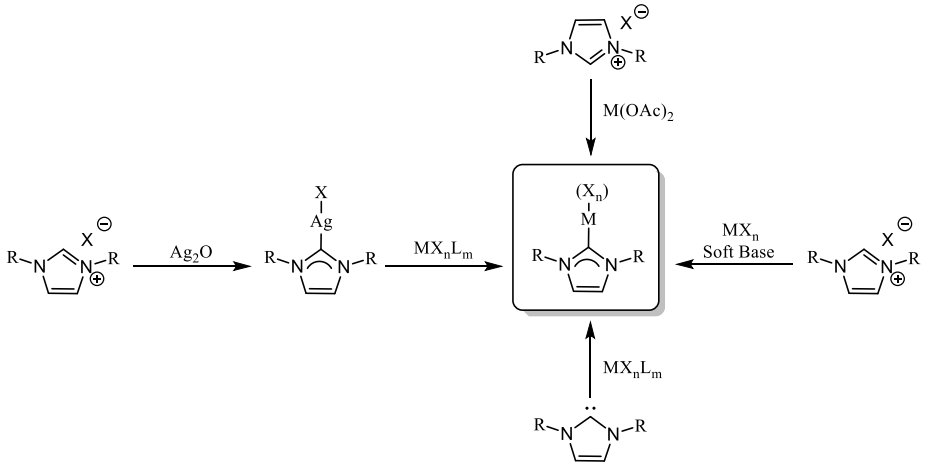
IV.3.1. General Aspects

Gold complexes bearing N-heterocyclic carbene (NHC) are currently one of the most promising classes of substances for drug research.[315-317] The story of neutral gold complexes started when Auranofin, a triethylphosphine gold(I) glucose-thiolate, was marketed in 1982 as an anti-rheumatic substance.[318] The oral availability of this substance represented a breakthrough in this area. Furthermore, the pharmacological behavior of this new class of metal complexes was shown to be different from that of the well-established platinum species ("Cisplatin"),[316,319] so that gold complexes became an important area of anti-cancer research. Inspired by the success of N-heterocyclic carbenes (NHC) in catalysis,[320] the idea was born to exchange the phosphine for an NHC ligand, which also acts as a strong sigma donor.[96,321-336] This replacement enhances the stability of the resulting complexes. An important feature of gold(I)-NHC complexes is their antimitochondrial activity,[96,324,325] which is important for the development of these substances as anti-tumor agents. The imidazole core allows facile variation of the *N*-substituents and of the backbone with the aim to modify properties such as lipophilicity, steric demand and donor strength of the NHC. Several complexes have been presented in the last few years and their biological activity is often impressive. Chloro(1-*p*-methyl-benzyl-3-methyl-imidazolin-2-ylidene)gold(I) was tested against protein tyrosine phosphatases and was found to exhibit potency in the low micromolar range.[293,326] A further target is the inhibition of thioredoxin reductase. In this direction gold(I) complexes of *N*-substituted benzimidazolin-2-ylidene with alkyl or benzyl groups[327] or of backbone-modified or *N*-arylated imidazolin-2-ylidenes[328] were also studied. The introduction of derivatives with additional amino groups[329] or with modifications in the aryl units of the backbone[94] has also been presented and their application as antitumoral drugs was described. Quite simple gold-NHC complexes, such as (imidazolin-2-ylidene) gold(I) chloride, were shown to have remarkable anti-cancer activity based on their antiproliferative properties against *cis*-platin-resistant cell lines.[330] Still new decorations of the imidazole core are developed and tested for biological activity.[331]

I decided to introduce the motif of 1,2,4-oxadiazole-substituted phenyl groups into the chemistry of imidazolin-2-ylidene-gold(I) complexes as a substituent at the nitrogen atom and to probe the effect on the biological activity. To the best of my knowledge, such structures have not yet been established in the chemistry of gold(I)-NHC complexes.

IV.3.2. Synthetic aspects

Several methods of generating gold(I) complexes have been described in the literature [321,328,332-341] (Scheme 62). The free carbene can be generated and subsequently reacted with a gold-containing precursor, usually [Au(DMS)Cl] or [Au(THT)Cl] (DMS = dimethyl-sulfide, THT = tetrahydrothiophene). However, this method requires special conditions because of the high reactivity of the free carbene, and not all free carbenes are sufficiently stable. Another approach uses silver[321,335] or copper[336,337] transmetallation protocols.



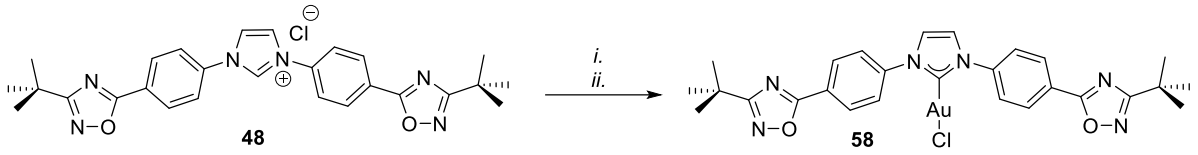
Scheme 62. General pathways in the synthesis of NHC-M complexes starting from imidazolium salts.

At first, a silver complex is generated from the reaction of the imidazolium salt with a weak silver base such as Ag_2O. One can either isolate these intermediates or use the reaction mixture for further reactions with an appropriate gold source, e.g. [Au(DMS)Cl] or [Au(THT)Cl]. We evaluated this method, but the yields were lower than those described in the literature and, considering that our ligands are not easy to synthesize, we tried to improve the protocol. Several protocols in the literature describe the *in situ* generation of the free carbene by using the base KO-*t*Bu to deprotonate the imidazolium salts in anhydrous THF, whereupon the gold source is added. Nevertheless, this reaction did not proceed cleanly in our hands, and several unidentified byproducts were formed, making the isolation of the desired gold compounds difficult. The byproduct formation raised questions regarding the stability of the free 1,2,4-oxadiazol-modified NHC, which might be the reason for the observed problems. It is reasonable to assume that these occur between the generation of free NHC and the addition of the gold source. Therefore, I added the imidazolium salts together with KO-*t*-Bu and [Au(DMS)Cl] to the reaction flask, and under an inert atmosphere, the solvent (dry THF) was added by syringe. The reaction was monitored by thin layer chromatography, and the results were very promising. After 15 minutes, the reaction was complete and no byproducts were observed, making the purification easy and the yields higher.

IV.3.3. Synthesis and structural characterization of 1,2,4-oxadiazole-Au(I)-NHC complexes

IV.3.3.1. Synthesis of Chloro(1,3-bis(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-1H-imidazolin-2(3H)-ylidene) gold(I) (58).

Using the silver transmetallation route, the 1,3-bis(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)imidazolium chloride (**48**) was transformed into the corresponding chloro(1,3-bis(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-1*H*-imidazolin-2(3*H*)-ylidene) gold(I) complex without any difficulties (Scheme 63). The yield could have been better (59%), but taking into consideration that all manipulations and purifications were made under normal atmospheric conditions and not into the inert atmosphere glovebox, can be that a part of the gold complex get destroyed.



48 58

Scheme 63. Synthesis of chloro(1,3-bis(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-1H-imidazolin-2(3H)-ylidene) gold(I) (58): i. Ag_2O, DCM, dark, rt; ii. Au(DMS)Cl, DCM.

The structure of the gold(I)-NHC complex **58** was supported by the IR, UV/Vis, ^{1}H NMR, ^{13}C NMR, MS (HR-ESI) and elemental analysis. The ^{1}H NMR resonance correlated to the carbenic proton of the precursor at 10.76 ppm disappears. In the ^{13}C NMR the C-Au signal is formed at 170.9 ppm. Although neutral NHC-Au(I) complexes usually give not good signals in the electrospray ionisation, compound **58** gave the corresponding (M + Na^+) HR-mass. The NHC-chlorogold(I) complex **58** was analyzed by X-ray diffraction analysis (Fig. 79). The coordination at gold, via the NHC carbon atom and a chloride ligand, is as expected close to linearity, with C-Au-Cl 175.96(11)° and bond lengths Au-C 1.990(4) and Au-Cl 2.2883(9) Å, which may be regarded as typical for NHC-Au-Cl complexes. A search of the Cambridge Structural Database [342] yielded 145 carbene-chlorogold(I) structures, with 189 values for these bond lengths; the average values were C-Au 1.985, Au-Cl 2.287 Å. The two aromatic rings subtend interplanar angles of 54 and 46° in opposite directions (unprimed and primed atoms respectively) to the NHC ring. The compound crystallizes as a chloroform disolvate, and both chloroform molecules are connected to the gold complex via weak hydrogen bonds (H98ΛCl1 2.68, H99ΛN2 2.35 Å). Despite the bulky NHC ligand, **58** forms inversion-symmetric dimers through aurophilic interactions with AuΛAu 3.2498(3) Å, a

feature which is often observed in the solid-state molecular structures of gold(I) complexes.[343-349]

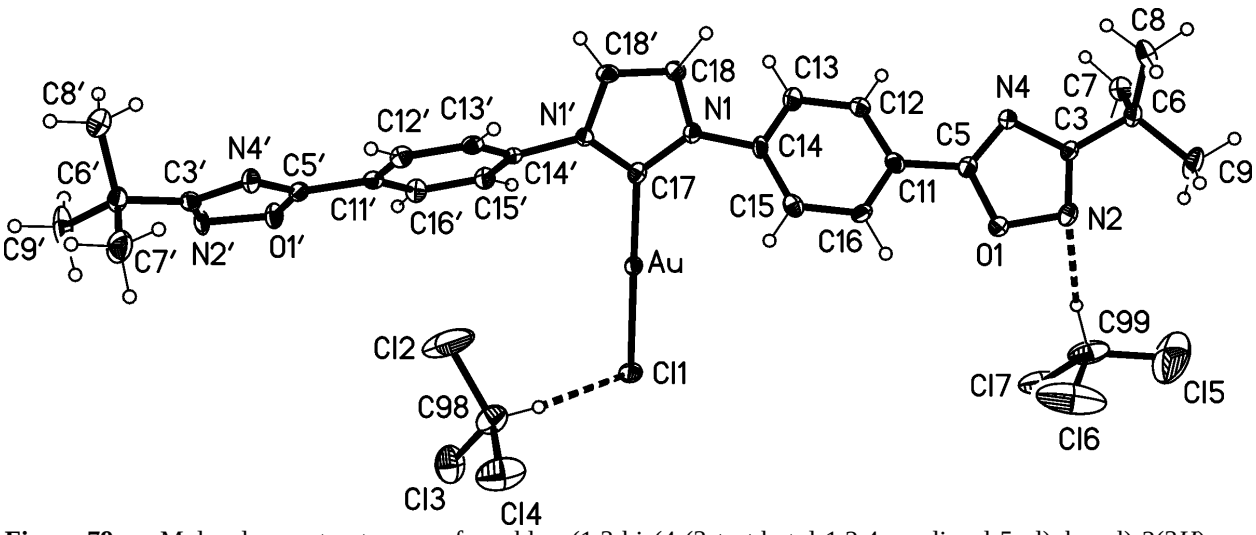


Figure 79. Molecular structure of chloro(1,3-bis(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-2(3*H*)-ylidene)gold(I) (**58**), including the two chloroform molecules. Atoms are drawn as 50% thermal ellipsoids. Weak hydrogen bonds are shown as dotted lines.

IV.3.3.2. Synthesis of Chloro(1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-methyl-1H-imidazolin-2(3H)-ylidene) gold(I) (59)

The methyl group is one of the most encountered features as second substituent in NHC-complexes due to its stability and the straightforward synthetic protocols. In Figure 80 are presented some examples of antitumoral NHC-Au(I)-Cl complexes that have the methyl group as second substituent on the imidazole ring. These compounds are reported to be active agains the ovarial tumor cell A 2780[330] or as selective thioredoxin reductase (TrxR).[327,328]

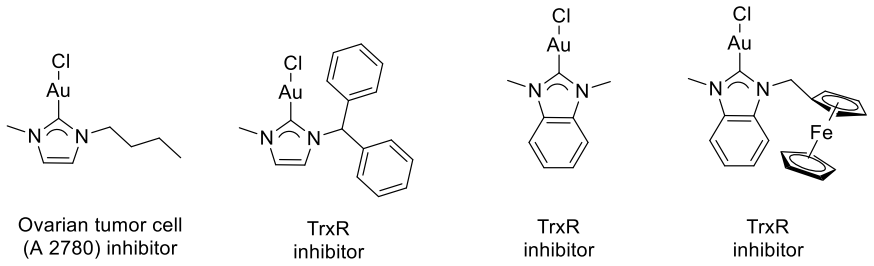
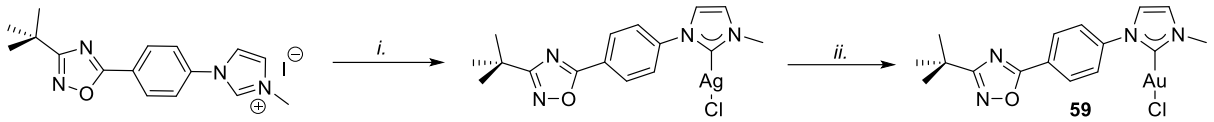


Figure 80. Examples of methyl substituted NHC-Au(I)-X complexes.

The unsymmetrical 1,2,4-oxadiazole-NHC-Au(I)-Cl complex having the methyl group as second substituent was obtained via the transmetallation route starting from the corresponding 1-(4-(3-*tert*-butyl-1,2,4-oxadiazole-5-yl)phenyl)-3-methyl-imidazolium iodide (Scheme 64).



Scheme 64. Synthesis of chloro(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-methyl-1H-imidazolin-2(3H)-ylidene) gold(I) (**59**): *i*. Ag_2O, DCM, dark, rt; *ii*. Au(DMS)Cl, DCM.

The new complex was isolated in acceptable yields (52%) over two steps and properly characterized by IR, UV/Vis, ^{1}H NMR, ^{13}C NMR, MS (EI) and elemental analysis. All analytics agree with the proposed structure. The formation of the C-Au bond is proven by the disappearence of the N-C*H*-N resonance at 9.98 ppm in the ^{1}H NMR and the shift of the C-carbenic resonance from 137.4 to 171.3 ppm.

The molecular structure of **59** is depicted in Figure 81 and shows the expected linear monocarbene gold(I) complex with bond lengths of Au-C_{NHC} 1.977(4), Au-Cl 2.2822(9) Å and a bond angle at gold of 172.61(10)°. The six-membered ring and the NHC ring subtend an interplanar angle of 40°.

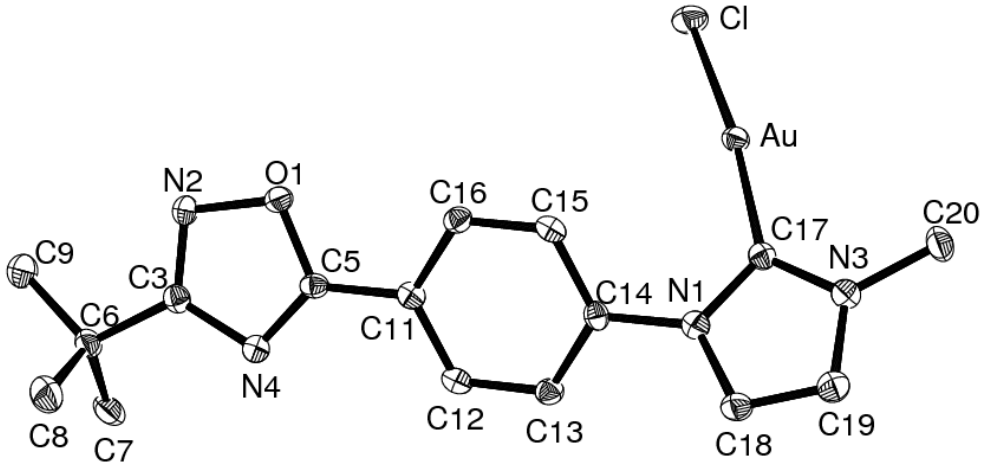


Figure 81. Molecular structure of chloro(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-methyl-1*H*-imidazol-2(3*H*)-ylidene)gold(I) (**59**). Atoms are drawn as 50% thermal ellipsoids. Hydrogen atoms are omitted for clarity.

IV.3.3.3. Synthesis of Bromo(1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(benzyl)-1H-imidazolin-2(3H) ylidene) gold(I) (60).

Benzyl substituent is also frequently encountered in the design of NHC-Au(I) complexes. Most of the reported compounds have been tested for their catalytic properties, but just a few examples of medicinal application are mentioned (Figure 82). Gust *et al.* prepared a series of 4,5-diarylimidazole-Au complexes and investigated them for antitumor activity.[94] All his complexes are thioredoxin reductase (TrxR) inhibitors. Another NHC-Au(I) complex TrxR inhibitor was described by Rubbiani *et al.*.[327]

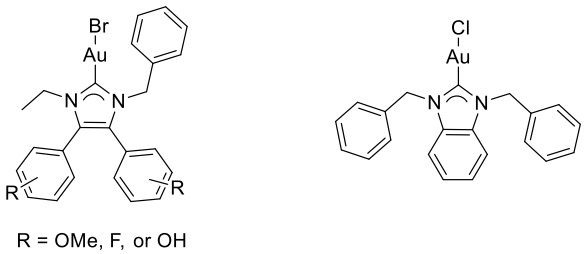
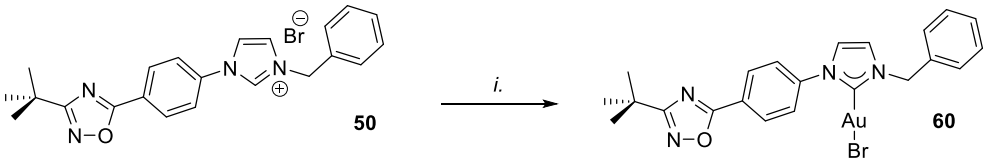


Figure 82. Reported benzyl substituted NHC-Au(I)-X complexes with antitumor activity.

The starting material for preparing bromo(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(benzyl)-1H-imidazolin-2(3H) ylidene) gold(I) was the corresponding imidazolium salt **50**. First attempts for the synthesis were a little bit disappointing. Using the silver transmetallation route, which was successful for the previously compounds, the isolated yield was less than 10%. After several struggles to improve the reaction protocol, I gave up and tried another established procedure. KO-*t*Bu was used as a base for the deprotonation of the imidazolium salt in THF and then the gold source was added. Unfortunately, this methodology was equally unsuccessful. Not only that the yield was low, also the compound could not be purified because many byproducts were present in the reaction mixture. Finally, the way of adding the ingredients to this reaction solved the problem. The imidazolium salt was mixed together with KO-*t*Bu and [Au(DMS)Cl] under nitrogen atmosphere and then dry THF was added. The reaction was very fast (5-15 min), clean and in very good yields (78%).



Scheme 65. Synthesis of bromo(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(benzyl)-1H-imidazolin-2(3H) ylidene) gold(I) (**60**): *i.* KO*t*Bu, [Au(DMS)Cl], THF, rt.

The IR, UV/Vis, ^{1}H NMR, ^{13}C NMR, MS (HR-ESI) and elemental analysis confirm the expected chemical structure. The C-Au resonance in the ^{13}C NMR spectroscopy is shifted from 137.8 ppm in the imidazolium salt to 173.7 ppm in the gold complex. Single crystals suitable for X-ray diffraction were obtained by slow diffusion of pentane into a solution of **60** in dichloromethane. The X-ray structure of compound **60** is shown in Figure 83. There are two independent molecules in the asymmetric unit, which are closely similar (r.m.s.d. 0.14 Å excluding terminal butyl carbons). The bond lengths C17-Au 1.989(8), 2.007(8) Å and Au-Br 2.3826(10), 2.3959(10) Å are in the normal range of NHC-Au-Br complexes; a CCDC search[361] gave 14 hits, 15 values for this fragment, with mean bond lengths of Au-C 1.985, Au-Br 2.394 Å. As usual, the angle around the metal centre C(17)-Au-Br is almost linear at

177.6(2), 176.9(2)°. The central NHC ring subtends an interplanar angle of 37° with the aromatic ring C11-16 but is almost perpendicular (79°) to the ring C21-26. Values are almost identical for the second molecule.

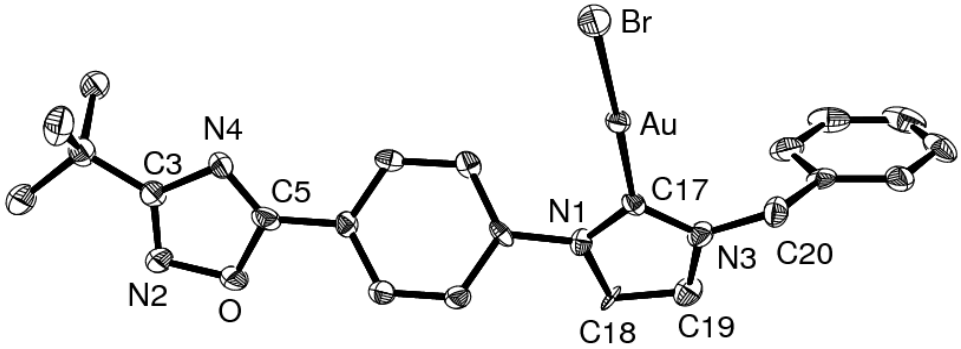


Figure 83. Molecular structure of **60**; one of two independent molecules. Atoms are drawn as 50% thermal ellipsoids. Hydrogen atoms are omitted for clarity.

The molecular packing involves broad layers of molecules, with molecule 1 occupying the region $x \approx ½$ (Fig. 83a) and molecule 2 at $x \approx 0$. The molecules are linked by short $H_{imidazole}$ΛAu contacts (2.86, 2.92 Å) and very long AuΛAu contacts (4.25, 4.21 Å). It is often a moot point whether HΛAu contacts represent genuine interactions or a merely a chance consequence of the sterically exposed nature of two-coordinate gold atoms.[350]

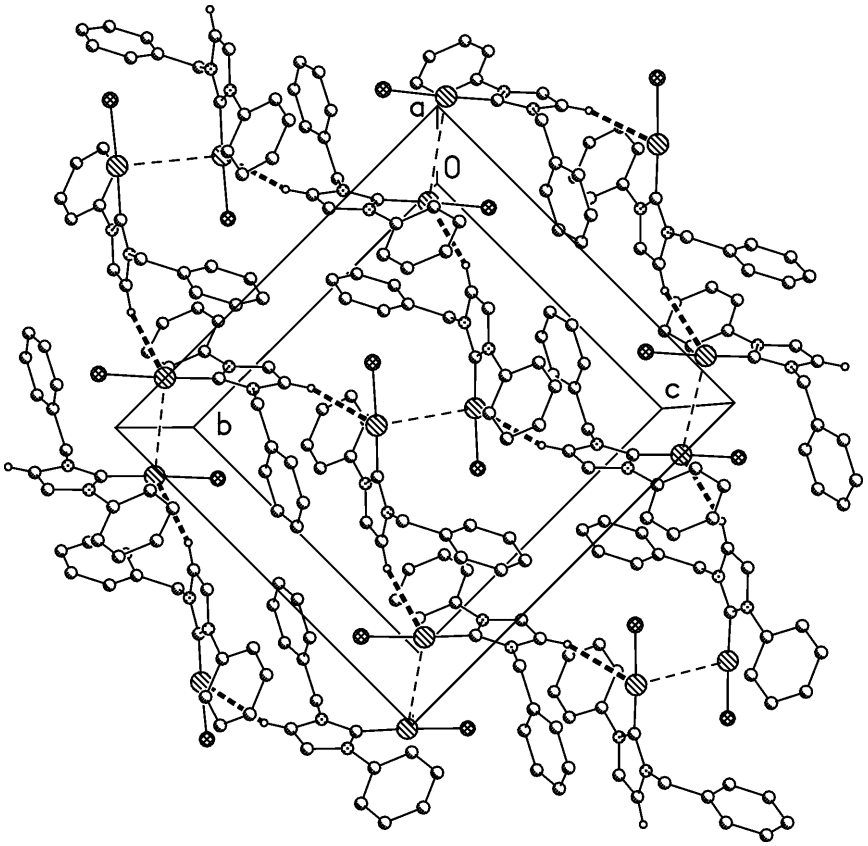


Figure 83a. Packing of compound **60**, first independent molecule only, viewed parallel to the *a* axis in the region $x \approx ½$. HΛAu and AuΛAu contacts are indicated by dashed lines. Other H atoms and the oxadiazole ring systems are omitted for clarity.

IV.3.3.4. Synthesis of Bromo(1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2-pyridine)-1H-imidazolin-2(3H)-ylidene)gold(I) (61)

NHC pincer-like complexes containing the 2-pyridine unit directly connected to the imidazole heterocycle are rarely reported.[267-269] Furthermore, no gold NHC complex of this type was found in the literature. However, the synthesized derivative **61**(bromo(1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2-pyridine)-1H-imidazolin-2(3H)-ylidene)gold(I)) could still be compared with the 1,3-benzimidazol-2-ylidene gold(I) complexes investigated by Dinda *et al.* despite the fact that the pyridine ligand is connected through a methylene bridge. The *in vitro* evaluation of the two complexes showed moderate activity against mouse melanoma (B16F10), human cervical carcinoma (HeLa) and human hepatocarcinoma (HepG2).[351]

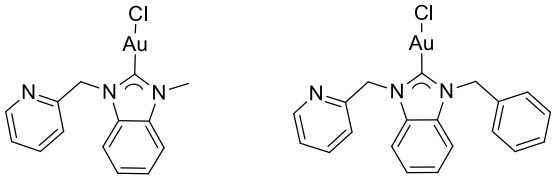
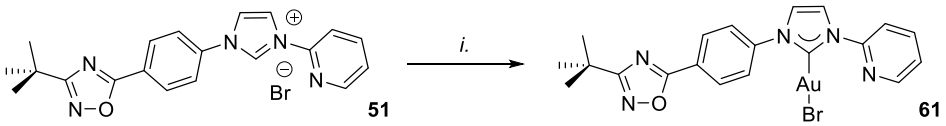


Figure 84. NHC-Au(I) complexes reported by Dinda *et al.*.

By reacting the pyridine-appended imidazolium salt **51** with KO*t*Bu and [Au(DMS)Cl] in THF in the absence of the light, the wanted bromo(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2-pyridine)-1H-imidazolin-2(3H)-ylidene)gold(I) was isolated in very good yield (85%) and accurately characterized.



Scheme 66. Synthesis of bromo(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2-pyridine)-1H-imidazolin-2(3H)-ylidene)gold(I) (61): *i.* KOtBu, [Au(DMS)Cl], THF, r.t.

Typical features of the asymmetric complex besides the pyridine signals are the imidazole protons (doublets at 8.03 and 7.48 ppm, J_{HH} = 2.0 Hz) and the C-Au resonance at 172.7 ppm. The high-resolution electron ionization mass spectrum (HR-EI) shows the predicted molecular peak (M 621.0433) along with the expected fragments given by the loose of Br (M-Br 542.0) and Br-Au (M-Br-Au 345.1). Single crystals suitable for X-ray diffraction were obtained by slow evaporation of a concentrated solution of **61** in dichloromethane.

The structure of compound **61**, which crystallizes as a dichloromethane solvate, is shown in Fig. 85. The Au-Br and C-Au bond lengths are 2.3962(3) Å and 1.987(3) Å respectively, with the usual linear coordination at gold, C17-Au-Br 177.88(8)°. The solvent

molecule is connected to the molecule of **61** by an acceptably linear contact (HΛBr 3.00 Å, C-HΛBr 166°) that may reasonably be classified as a "weak" hydrogen bond. This ensemble also includes an intramolecular Cl2ΛO contact of 3.34 Å, which may be recognized in Fig. 85a although it is not drawn explicitly. The six-membered rings C11-16 and N5, C20-24 subtend interplanar angles of 47(4)° and 51(4)° respectively with the central NHC ring and are approximately perpendicular to each other. The pyridinic nitrogen atom N5 is clearly identifiable by its shorter ring bonds [1.328(4), 1.343(4) Å] and its narrow ring angle of 115.9(3)°.

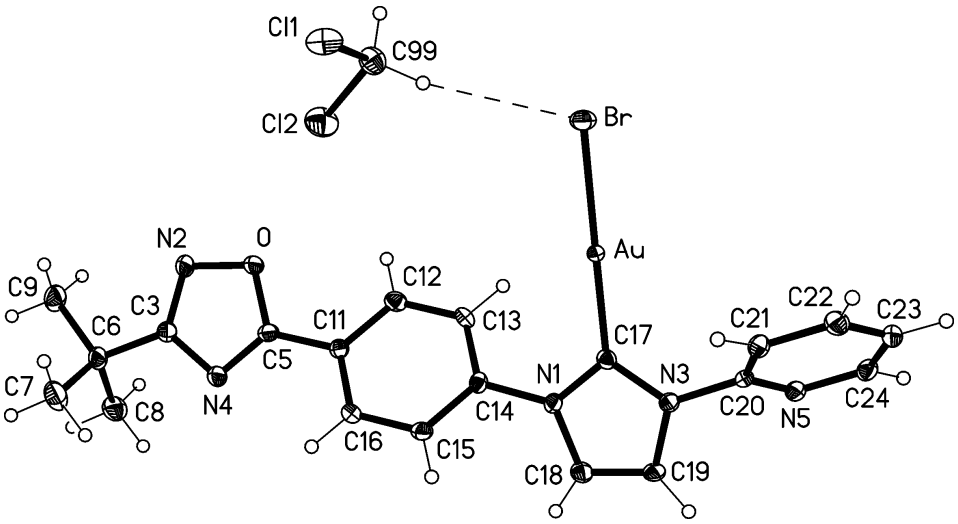


Figure 85. Molecular structure of bromo(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2-pyridine)-1*H*-imidazol-2(3*H*)-ylidene)gold(I) (**61**) as its dichloromethane solvate. Atoms are drawn as 50% thermal ellipsoids. The dashed bond is a weak hydrogen bond.

The most striking intermolecular contact is the aurophilic interaction AuΛAu of 3.3373(3) Å, forming centrosymmetric dimers. These are further linked via weak hydrogen bonds H19ΛN5 (2.44 Å, 148°) and H12ΛO (2.45 Å, 156°) to form a layer structure parallel to the planes (110) (Fig. 85a).

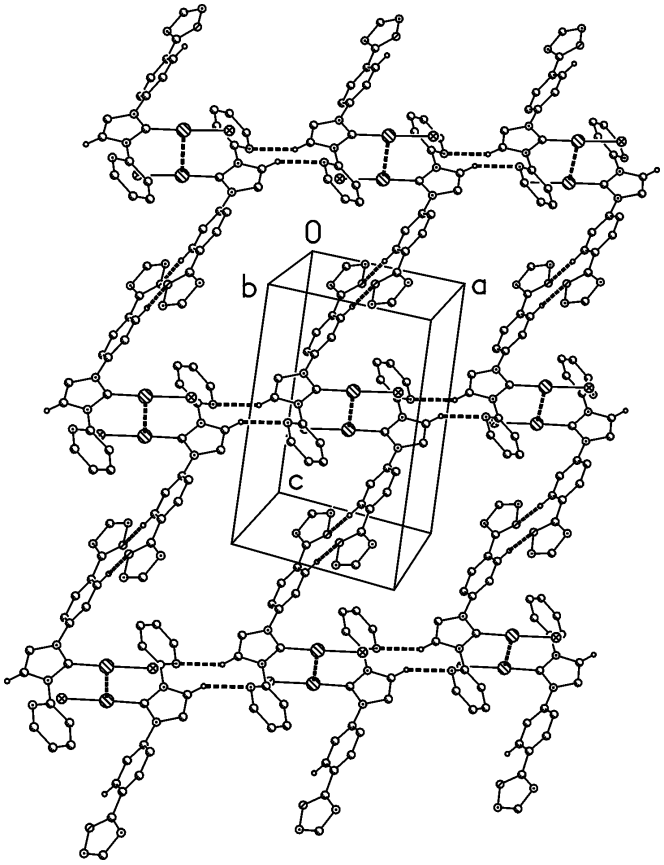


Figure 85a. Packing diagram of **61** (solvent omitted) with view direction approximately parallel to the *b* axis. Dashed bonds indicate weak hydrogen bonds or AuΛAu interactions.

IV.3.3.5. Synthesis of Chloro(1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(9-methyl-anthracene)-1H-imidazolin -2(3H) -ylidene)gold(I) (62)

The Au(I)-NHC-Cl complex bearing anthracenyl substituent was investigated by Citta *et al*. (Fig. 86). The fluorescent complex was tested for cytotoxicity against normal and tumor cell lines. The results showed good activity, but noselectivity.[272]

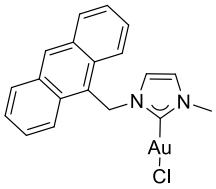
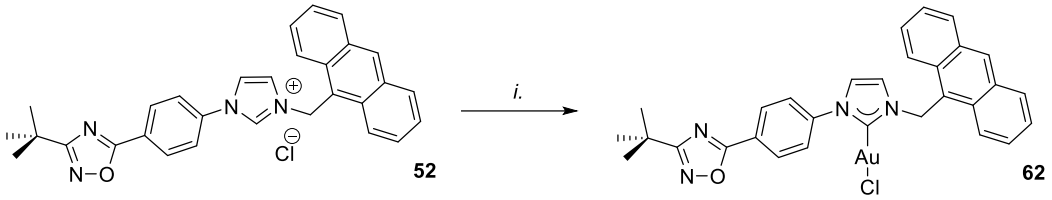


Figure 86. Anthracenyl NHC-Au(I) complex reported by Citta *et al.*.

Starting from 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(9-methyl-anthracene)-imidazolium chloride (**52**), it was possible to obtain the desired gold complex in 63% yield using the synthetic protocol developed previously (mix all ingredients and add the solvent at the end). The new 1,2,4-oxadiazole-antracenyl NHC-Au(I) complex was fully characterized by IR, UV/Vis, ^{1}H NMR, ^{13}C NMR, MS (HR-ESI) and elemental analysis.

Some specific features of the molecule are the C-Au resonance at 170.6 ppm, the two backbone proton signals of the imidazole ring as two doublets (7.01 and 6.49 ppm) with a J_{HH} = 2 Hz and the methylene bridge between the imidazole and anthracenyl as a singlet at 6.32 ppm.



Scheme 67. Synthesis of chloro(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(9-methyl-anthracene)-1H-imidazolin -2(3H) -ylidene)gold(I) (**62**): i. KOtBu, [Au(DMS)Cl], THF, r.t.

Single crystals suitable for X-ray diffraction were obtained by slow evaporation of a concentrated solution of **62** in dichloromethane. The X-ray structure of compound **62** is shown in Fig. 87. The coordination at gold is described by the bond lengths and angles C_{NHC}-Au 1.982(2), Au-Cl 2.2719(5) Å, C_{NHC}-Au-Cl 177.35(6)°. The central NHC ring subtends interplanar angles of 47° to the ring C11-16 and 63° to the anthracenyl system.

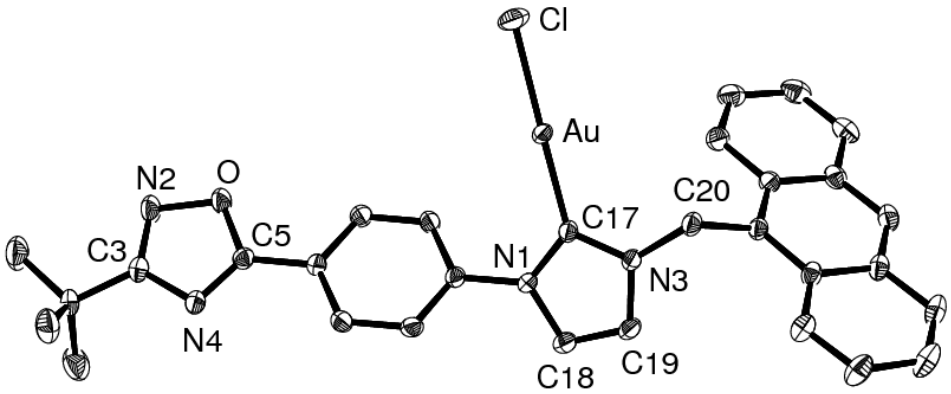


Figure 87. Molecular structure of chloro(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(9-methyl-anthracene)-1*H*-imidazol-2(3*H*)-ylidene)gold(I) (**62**). Atoms are drawn as 50% thermal ellipsoids. Hydrogen atoms are omitted for clarity.

The packing of **62** involves ribbons of molecules parallel to [101] linked by the "weak" but short hydrogen bonds H20AΛCl 2.71 and H16ΛO 2.41 Å and a borderline AuΛAu contact of 3.977 Å (Fig. 87a).

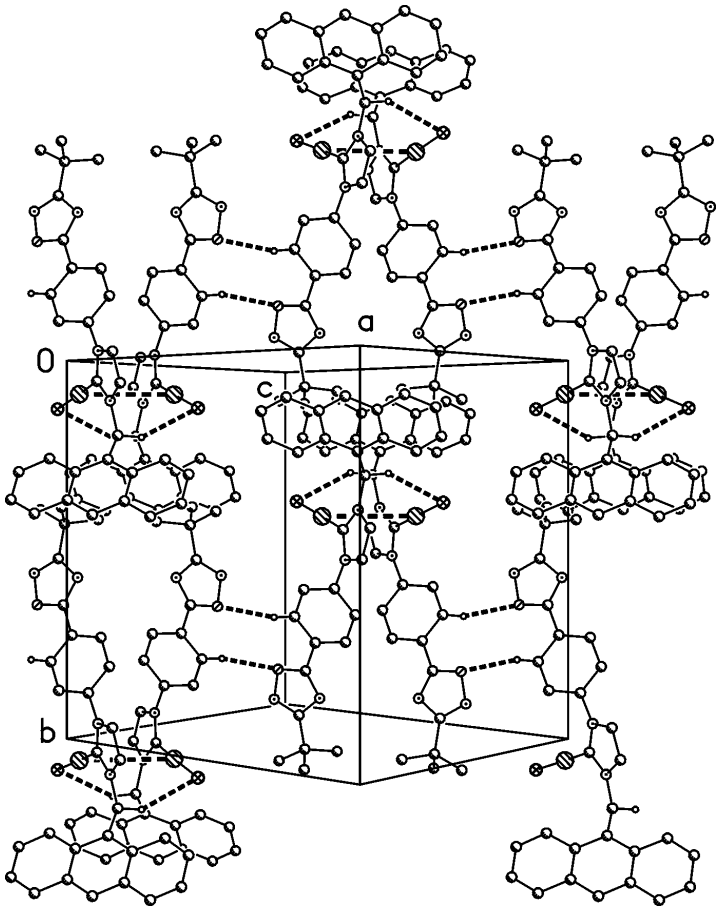
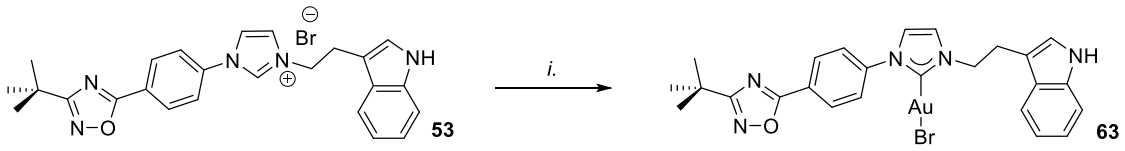


Figure 87a. Packing of compound **62**, showing two ribbons parallel to [101]. Hydrogen bonds and AuΛAu contacts are indicated by dashed lines. Other H atoms are omitted for clarity.

IV.3.3.6. Synthesis of Bromo(1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-((2-ethyl)indole)-1H-imidazolin-2(3H)-ylidene)gold(I) (63)

To the best of my knowledge this type of indole containing NHC-Au complexes has not yet been reported in the literature. Bromo(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-((2-ethyl)indole)-1*H*-imidazolin-2(3*H*)-ylidene)gold(I) (**63**) was prepared starting from the imidazolium salt analog **53** using the procedure described previously.



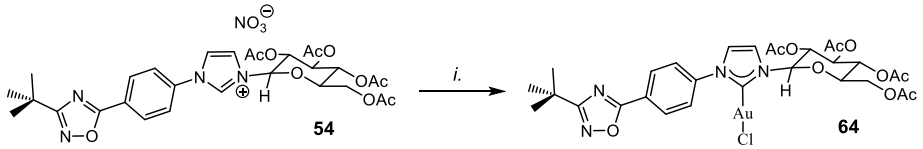
Scheme 68. Synthesis of bromo(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-((2-ethyl)indole)-1H-imidazolin-2(3H)-ylidene)gold(I) (**63**): *i.* KOtBu, [Au(DMS)Cl], THF, r.t.

The chemical structure of the new complex was undoubtedly confirmed by IR, UV/Vis, ^{1}H NMR, ^{13}C NMR, MS (EI) and elemental analysis. The characteristic N*C*N-Au resonance was observed in the ^{13}C NMR spectrum at 173.7 ppm. Furthermore the indole-N*H*

signal and the ethylene bridge are found at 8.31 ppm (singlet) and 4.5 and 3.34 ppm (triplet), respectively; the other units of the molecule show no unusual resonances in ^{1}H NMR spectrum.

IV.3.3.7. Synthesis of Chloro(1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2,3,4,5-tetra-O-acetyl-D-glucopyranosyl)-1H-imidazolin-2(3H)-ylidene)gold(I) (64)

The 1,2,4-oxadiazole-carbohydrate gold(I)-NHC complex **64** was prepared from the coresponding imidazolium salt **54** in good yield (52%) and purity (Scheme 69).

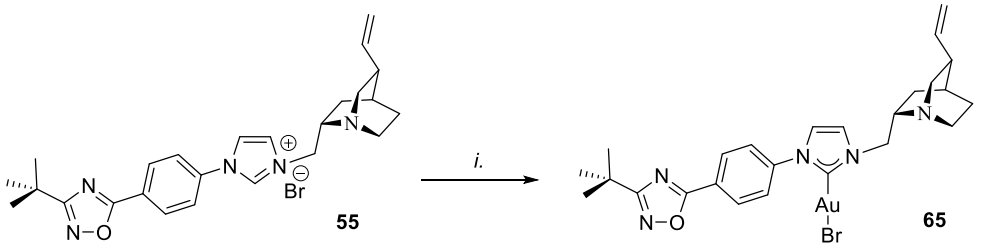


Scheme 69. Synthesis of chloro(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2,3,4,5-tetra-*O*-acetyl-*D*-glucopyranosyl)-1*H*-imidazolin-2(3*H*)-ylidene)gold(I) (**64**)): *i.* KOtBu, [Au(DMS)Cl], THF, r.t.

The structure of the compolex was confirmed by IR, NMR, HR-ESI and elemental analyses.

IV.3.3.8. Synthesis of Bromo(1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCI)-1H-imidazolin-2(3H)-ylidene)gold(I) (65)

The complex **65** was prepared starting from its precursor, 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCI)-imidazolium bromide (**55**), in acceptable yield (41%) and good purity. The lack of chromophores along with the basicity of the molecule made the purification rather tedious. The new complex was thoroughly characterized by IR and NMR spectroscopy as well as mass spectrometry. The ^{1}H NMR spectrum of this unique complex misses the specific N-C*H*-N resonance from 9.26 ppm. Furthermore, the two unsymmetrical imidazole protons revealed themselves at 7.51 and 7.28 ppm as doublets with a coupling constant of J_{HH} = 1.98 Hz. At the same time the ^{13}C NMR spectrum supported the formation of the metal-carbene bond (N*C*N-Au) with a downfield resonance at 181.8 ppm. The structural assignment was further strengthened by EI-mass spectrometry (693.1 / 695.1 M^{+}100) and elemental analyses.



Scheme 70. Synthesis of bromo(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCI)-1H-imidazolin-2(3H)-ylidene)gold(I) (**65**): i. KOtBu, [Au(DMS)Cl], THF, r.t.

The molecular structure of the enantiomerically pure compound **65** is shown in Fig. 86. The dimensions around the gold atom are C17-Au 1.994(3), Au-Br 2.4010(4) Å, and C17-Au-Br 177.42(10)°. The QCI substituent shows a C28-C29 bond length of 1.319(5) Å, consistent with a CC double bond.

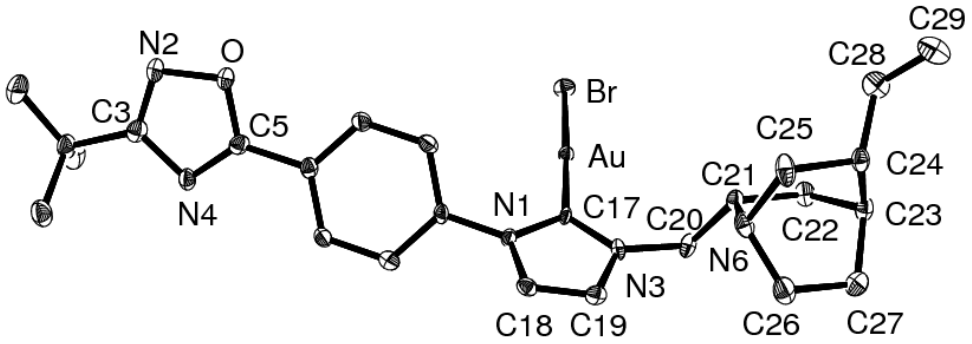
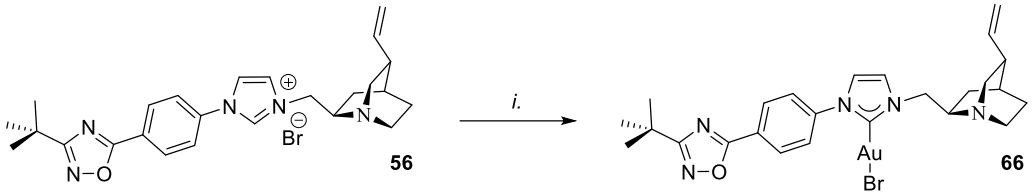


Figure 88. Molecular structure of bromo(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCI)-1*H*-imidazol-2(3*H*)-ylidene)gold(I) (**65**). Atoms are drawn as 50% thermal ellipsoids.

The molecular packing involves an aurophilic interaction of 3.4586(2) Å between neighboring molecules related by the 2_1 operator parallel to the *a* axis, thus leading to a chain of molecules with Au-Au-Au angle 163.79(2)°. Otherwise the packing shows very few short contacts.

IV.3.3.9. Synthesis of Bromo(1-(4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCD)-1H-imidazolin-2(3H)-ylidene)gold(I) (66)

The other enantiopure QCD-NHC-Au(I) complex **66** was synthesised in a similar manner starting from the corresponding 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCD)-imidazolium bromide (**56**). The isolated yield was slightly better (47%) than that forthe QCI analogue (41%).

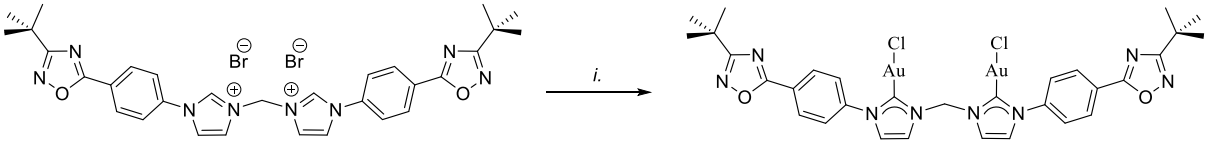


Scheme 71. Synthesis of bromo(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCD)-1H-imidazolin-2(3H)-ylidene)gold(I) (**66**): *i*. KOtBu, [Au(DMS)Cl], THF, r.t.

Peculiar NMR spectroscopic aspects of the new gold complex involve also the disappearance of the C_2 proton belonging to the imidazolium salt **56** (10.96 ppm) in the ^{1}H NMR spectrum and a strong shift of the N-*C*-N resonance (from 137.6 to 181.9 ppm) in the ^{13}C NMR spectrum. The EI-mass spectrometry and elemental analyses further validate the proposed molecular structure.

IV.3.3.10. Synthesis of Dichloro{1,1′-[5-(phenyl)-3-(tert-butyl)-1,2,4-oxadiazole]-3,3′-methylene-diimidazolin-2,2′-diylidene}digold(I) (67).

Following the silver transmetallation route, starting from 1,1′-[4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl]-3,3′-methylene diimidazolium bis(bromide) (**57**) it was possible to isolate compound **67**, albeit in low yield (35%).



Scheme 72. Synthesis of dichloro{1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylene-diimidazolin-2,2′-diylidene}digold(I) (**67**): *i*. KOtBu, [Au(DMS)Cl], THF, r.t.

The generation of the Bis-Au(I) carbene complex was established by IR, NMR, HR-ESI and elemental analyses. The disappearance of the carbenic acidic protons at 10.56 ppm in the ^{1}H NMR spectra and appearance of the NCN-Au signal at 171.9 ppm in the ^{13}C NMR spectrum indicate the formation of a coordinative bond between the dicarbene and the gold atoms. The structure is also confirmed also by HR-ESI and elemental analyses.

IV.3.4. NMR features of the Au(I)-NHC-compounds

The formation of the gold(I) complexes is proven beyond any doubt by NMR spectroscopy. The disappearance of the N-C*H*-N resonance in the ^{1}H NMR along with the shifting in the up-fielded area (170.3-181.9 ppm) of the resonance for the carbene carbon atoms are strong hints that the imidazolium salts were deprotonated and the C-Au bond was established. These values are in the range of reported values for Au(I)-NHC complexes bearing a C-Au-X (X = halide) fragment.

Table 5. Notable features in the ^{1}H NMR and ^{13}C NMR spectra of the imidazolium salts and the corresponding NHC-Au(I) complexes.

NHC-Au(I) complex	δ_C *[ppm]* (NHC-Au) [a)]	Imidazolium salt	Solvent	δ_H *[ppm]* (NHC*HX)	δ_C *[ppm]* (NHC*HX)	$\Delta\delta_C$
58	170.9	**48**	DMSO-d6	10.76	b)	
59	171.3	**49**	DMSO-d6	9.98	137.4	33.9
60	173.7	**50**	DMSO-d6	10.41	137.8	35.9
61	172.7	**51**	DMSO-d6	10.88	137.6	35.1
62	170.6	**52**	DMSO-d6	10.49	147.2	23.4
63	173.7	**53**	DMSO-d6	10.09	138.4	35.3
64	171.0	**54**	$CDCl_3$	11.09	137.8	33.2
65	181.8	**55**	MeOH-d4	9.26	139.3	42.5
66	181.9	**56**	$CDCl_3$	10.96	136.6	45.3
67	171.9	**57**	DMSO-d6	10.56	b)	

a) ^{13}C NMR spectra were recorded using $CDCl_3$ as solvent. b) The insufficient solubility prevented the collection of a meaningful ^{13}C NMR data.

IV.3.5. FT-IR of the NHC-compounds

Haque *et al.*[312] employed FT-IR to determine the transformation of imidazolium salts to the corresponding Ag(I) derivatives. Therefore, I also recorded the FT-IR spectrum of my compounds **48** to **67**. In general, it can be concluded that the FT-IR spectra of all pairs of imidazolium salt and corresponding Au(I) or Ag(I) complex look very similar. This is reasonable since there are only minor differences in the functional groups which can be characterized by IR spectroscopy. However, a notable feature in the IR spectra of the

imidazolium salts **49** to **57** is a strong and sharp stretch in the region of $1/\lambda$ = 1547-1555 cm^{-1}. Only for compound **48** this stretch is shifted to $1/\lambda$ = 1524cm^{-1}. Once the metal bond is formed this intense vibration significantly diminishes, whereas the other vibrations remain nearly uneffected. We therefore propose that this stretch may be attributed to the -HC=N- functionality.

IV.3.6. *In vitro* anti-tumor activity of 1,2,4-oxadiazole-containing NHC-Au(I) complexes towards human tumor cell lines

The *in vitro* anti-tumor activity of the compounds **58-67** was assessed in a panel of 12 human tumor cell lines using a monolayer cell survival and proliferation assay. As shown in Figure 89, excellent potency with mean IC_{50} values <0.1 µM was detected for 6 compounds, namely **61** (mean IC_{50} = 0.012 µM), **63** (0.019 µM), **64** (0.020 µM), **65** (0.049 µM), **60** (0.053 µM) and **66** (0.058 µM). In addition, good potency was detected for **62** (mean IC_{50} = 0.208 µM) and at a lower level for **58** (IC_{50} in the range from 1 to 10 µM).

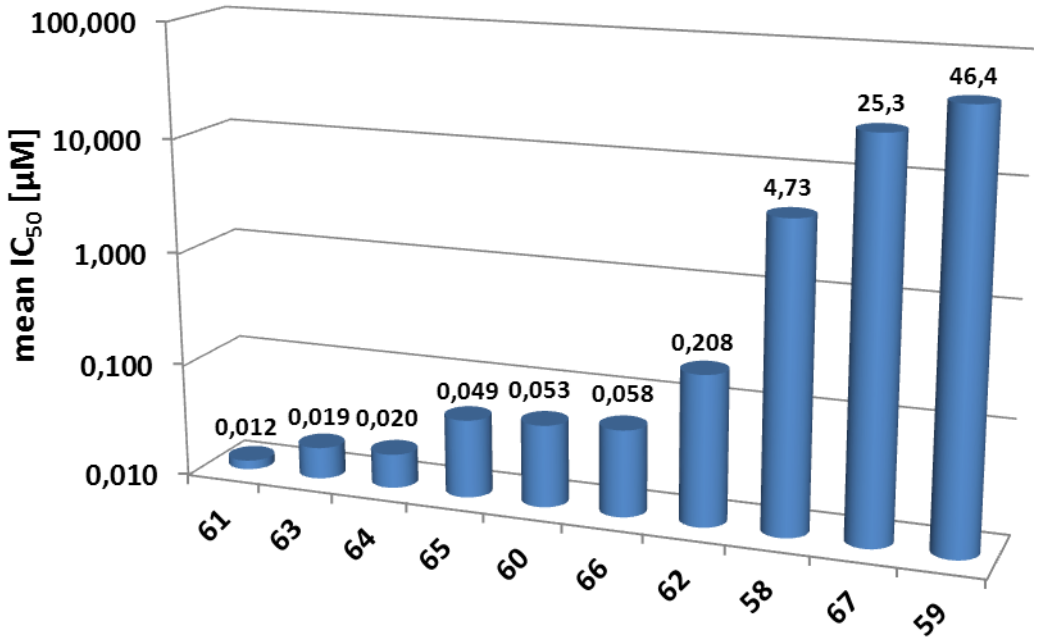


Figure 89. In vitro anti-tumor activity of compounds **58-67** in a panel of 12 human tumor cell lines (mean IC_{50} values).

As shown in Figure 90 (IC_{50} heatmap presentation), the six most active compounds exhibit a selective activity profile across the 12 cell lines tested, with the cell lines GXF 251 (gastric cancer), LXFA 629 (lung cancer), MAXF 401 (mammary cancer), 22RV1 (prostate cancer and UXF 1138 (cancer of the uteri body) showing above-average sensitivity, while OVXF 899 (ovarian cancer) and RXF 486 (renal cancer) appeared to be resistant. With respect to compound **61**, strong selective activity was evident by individual IC_{50} values from

0.003 µM (MAXF 401) to 0.871 µM (RXF 486), corresponding to a 290-fold difference between the most sensitive and most resistant cell line. Importantly, the activity profiles of these six compounds were quite similar to each other, indicating a similar mode of action.

		Cell lines												
Compound	Unit	CXF HT-29	GXF 251	LXFA 629	LXFL 529	MAXF 401	MEXF 462	OVXF 899	PAXF 1657	PRXF 22Rv1	PXF 1752	RXF 486	UXF 1138	geomean IC50 [µM]
61	µM	0,006	0,005	0,003	0,009	0,003	0,006	0,396	0,007	0,004	0,015	0,595	0,004	**0,012**
63	µM	0,017	0,006	0,005	0,018	0,003	0,009	0,564	0,014	0,009	0,024	0,871	0,006	**0,019**
64	µM	0,012	0,005	0,006	0,017	0,003	0,011	0,529	0,017	0,010	0,033	1,031	0,007	**0,020**
65	µM	0,030	0,029	0,017	0,042	0,009	0,024	0,914	0,040	0,023	0,071	1,462	0,017	**0,049**
60	µM	0,037	0,056	0,027	0,035	0,008	0,023	1,472	0,044	0,022	0,079	1,062	0,011	**0,053**
66	µM	0,034	0,058	0,019	0,049	0,008	0,035	1,066	0,039	0,022	0,064	2,389	0,019	**0,058**
62	µM	0,327	0,219	0,131	0,075	0,074	0,053	2,483	0,073	0,239	0,515	4,130	0,026	**0,208**
58	µM	15,395	2,280	7,371	3,917	2,246	2,484	20,212	4,254	n.d	5,313	3,046	3,377	**4,73**
67	µM	79,101	37,050	45,200	10,451	11,525	39,739	44,954	10,229	n.d.	29,987	18,186	16,782	**25,3**
59	µM	92,246	44,707	67,544	36,323	32,825	34,460	80,443	35,766	n.d.	46,476	31,885	44,366	**46,4**

1/32 1/16 1/8 1/4 1/2 1 2 4 8 16 32 -fold mean IC_{50}

sensitive cell lines — resistant cell lines

Figure 90. Heatmap presentation of individual IC_{50} values for compounds **58**-**67** in a panel of 12 human tumor cell lines.

Figure 91 exemplarily shows the IC_{50} mean graph presentation for the two most active compounds **61** and **63** and also emphasizes the similar activity pattern. Good tumor selectivity was further indicated for **62**, while all other compounds showed a lower level of selectivity or was not active at all.

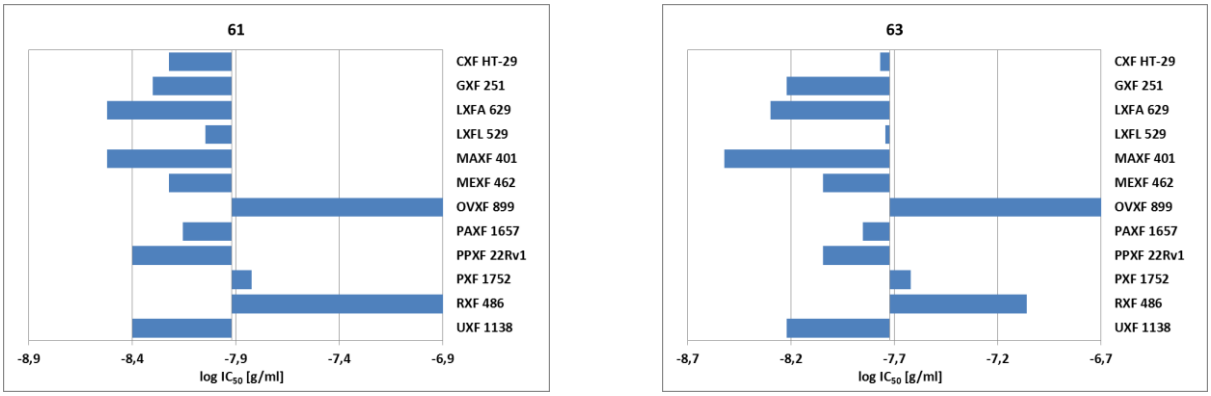


Figure 91. IC_{50} mean graph presentation for compounds **61** and **63**.

Comparing the in vitro activity of the compounds with established platinum compounds approved for cancer therapy revealed a markedly higher potency (Figure 92). Five platinum compounds of the first and second generation exhibited geometric mean IC_{50} values across the 12 cell line panel in the range from 0.96 µM (Satraplatin) to 11.68 µM (Carboplatin). In particular **61**, **63**, **64**, **65**, **60**, **66** and **62** were clearly more potent than the approved reference platinum compounds. Further investigation on the mode of action and *in vivo* efficacy studies are warranted for these highly attractive anticancer compounds.

compound	unit	Cell lines: CXF HT-29	GXF 251	LXFA 629	LXFL 529	MAXF 401	MEXF 462	OVXF 899	PAXF 1657	PRXF 22Rv1	PXF 1752	RXF 486	UXF 1138	Geom. mean IC_{50} [µM]
Satraplatin	µM	2,07	1,11	1,32	0,82	0,27	0,75	0,57	1,11	0,46	3,07	2,83	0,47	**0,96**
Tetraplatin	µM	1,29	0,43	18,27	0,84	0,40	2,33	0,46	2,69	0,70	8,10	1,05	2,11	**1,49**
Oxaliplatin	µM	1,28	1,55	12,13	3,37	0,60	5,32	0,21	4,77	0,71	15,34	0,69	3,36	**2,08**
Cisplatin	µM	14,55	4,97	4,71	0,83	0,68	0,73	2,54	7,62	3,57	38,58	8,79	2,77	**3,80**
Carboplatin	µM	26,16	9,19	19,65	4,69	1,98	4,04	8,01	36,61	9,99	52,31	27,68	8,53	**11,68**

1/32 1/16 1/8 1/4 1/2 1 2 4 8 16 32 -fold mean IC_{50}

sensitive cell lines — resistant cell lines

Figure 92. Heatmap presentation of individual IC_{50} values for approved platinum compounds in a panel of 12 human tumor cell lines (historical data of Oncotest).

IV.4. Synthesis and structural characterization of silver(I)-NHC complexes with 1,2,4-oxadiazole substituents

IV.4.1. General Aspects

In the last decade a large number of NHC imidazolium salts and their corresponding silver(I) complexes have been investigated as potential candidates against tumor growth. Thinking in this direction, in order to develop new bioactive silver(I)-NHC complexes, it was necessary to prepare a large number of functionalized and non-functionalized imidazole-based NHCs which could be tested for antitumor activity.

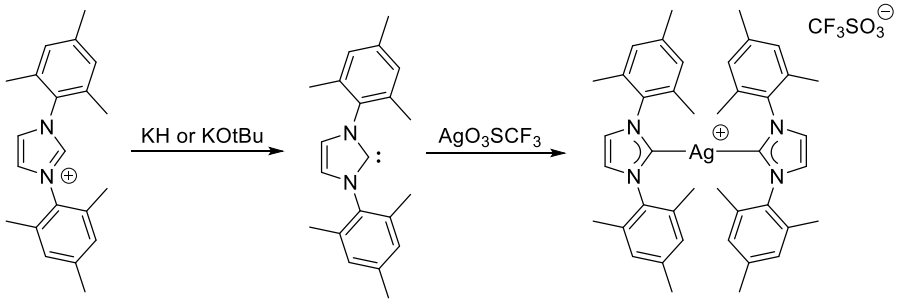


Figure 93. Synthesis of the first Ag(I)-NHC complex.

Arduengo *et al.* reported the first synthetic route of an Ag(I)-NHC complex (Fig. 93).[352] The method used involved the generation of the free carbene after deprotonation of the imidazolium salt in the presence of a base. Nevertheless this method is limited by the difficulties that can occur in the process of generating and handling the free carbenes. It is well known that free carbenes are sensitive against air, humidity and high temperatures.[249,353] For this reason the most common method used now-days in the synthesis of Ag(I)-NHC complexes is the *in situ* deprotonation of imidazolium salts precursors with basic silver agents

(Ag_2O, $Ag_2(OAc)$, Ag_2CO_3).[248,354,355] From all this methods, the most accessible way to generate the Ag(I) complexes involves the use of Ag_2O.[356-358] This reaction can be performed at room temperature using a large number of solvents, including water.[359-361] Silver(I) NHC complexes can be used as carbene transfer reagents because of their labile silver-carbene bond. Lin *et al*.employed this method for the first time in the synthesis of palladium and gold complexes[348,354] and many transitional metal complexes were generated using this method thereafter. The application of silver complexes as antitumorals was not intensively explored until recent time. A series of silver carboxylates and phosphine derivatives have been shown to possess *in vitro* activity against several tumor cell lines.[362] Some Ag(I)-NHC complexes have been described as possible antitumor agents against ovarian and breast cancer cell lines,[363] other saturated and unsaturated NHC-Ag(I) chloride derivatives showed better cytotoxicity than the marketed cisplatin against several tumor cell lines.[361] Another good example in this sense are the Ag(I)-NHC acetate complexes which have been reported recently to exhibit antitumor activity against one of the kidney cancer cell line.[364]

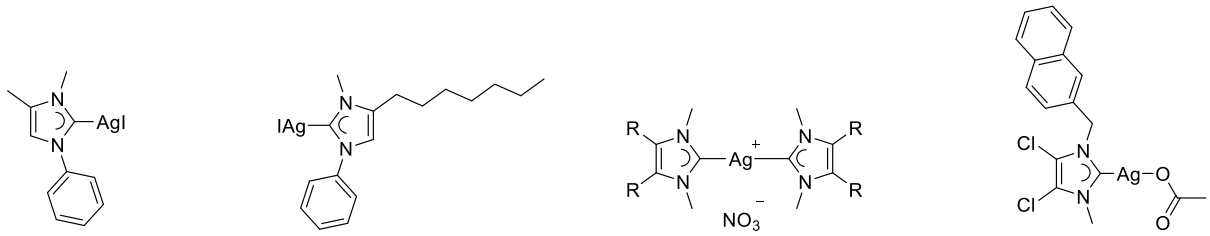


Figure 94. Examples of Ag(I)-NHC complexes with cytotoxic activity.

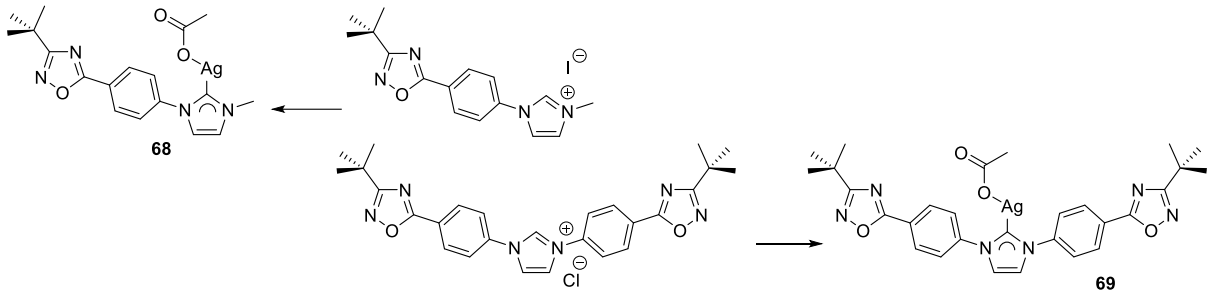
This reported results represent a good motivation ground for the development of novel synthetic Ag(I)-NHC chemistry as antitumoral drugs.

IV.4.2. Synthesis of 1,2,4-oxadiazole silver(I) NHC-complexes

Taking into consideration earlier discussions regarding the antitumor activity of Ag(I)-NHC complexes I built-up and investigated the antitumor activity of a small library of new compounds in which Ag(I)-NHC complexes contained the 1,2,4-oxadiazole motif. The bulky ligand confers higher stability to light and humidity (several days) to these molecules, making them possible candidates as antitumor agents.

IV.4.2.1. Synthesis of neutral 1,2,4-oxadiazole silver(I) NHC-complexes

The silver acetate complexes **68** and **69** belong to the Ag(I)-NHC complexes that were designed and investigated are. These two compounds were generated according to literature-based procedure.[364] This class of silver complexes is considered to be more stable than the corresponding silver salts, especially in the *in vivo* conditions, where, the free silver ions can interact with other molecules and generate secondary complexes in the bloodstream. The synthetic route of the silver acetate complexes starting from the imidazolium salts **48** and **49** is depicted in Scheme 73.



Scheme 73. Synthesis of Ag(I)-NHC acetate complexes **68** and **69**.

The Ag(OAc) used in double molar excess, deprotonated *in situ* the imidazolium precursors and attacked in the same time the free carbene developed. The two new silver acetate complexes were isolated in good yields, characterized and tested for *in vitro* antitumor activity. The only noteworthy feature in their ^{1}H NMR spectrum is the disappearance of the imidazolium proton signals from 10.7 and 9.9 ppm respectively. The ^{13}C NMR spectrum shows the formation of new Ag-C bond at 179.04 ppm and 179.8 ppm, respectively, and the comittant disappearance of the resonance around 137 ppm.

Apart from compounds **68** and **69** it was planned to produce other neutral Ag(I)-NHC complexes bearing more sophisticated substituents (Figure 95) starting from their corresponding imidazolium salts.

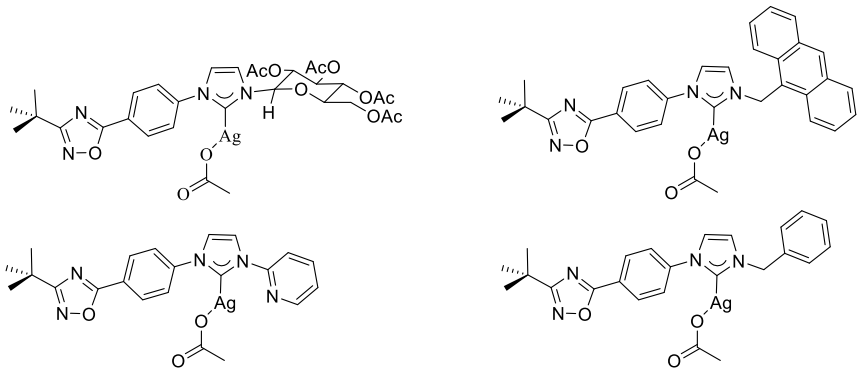


Figure 95. Other targeted Ag(I)-NHC acetate complexes.

Unfortunately these four complexes could not be isolated in pure form regardless of the amount of AgOAc added, the reaction time or temperature. Under all conditions investigated the conversion was never complete reaching a maximum of 50% based on the integrations in the ^{1}H NMR spectrum. Moreover, even if the new complexes were formed, they appeared to be unstable and I could not isolate a pure sample for characterization and biological testing. After many struggles and attempts this synthetic target was no longer pursued, but the idea to generate Ag(I)-NHC complexes with these four ligand incorporated was still in my mind.

IV.4.2.2. Synthesis of cationic 1,2,4-oxadiazole silver(I) NHC-complexes

Inspired by the reported work of Siciliano *et al.*,[365] the strategy was to build-up novel Ag(I)-NHC complexes having the imidazole ring which carries a 1,2,4-oxadiazole motif in 4-position and benzyl, 2-pyridine, anthracene and carbohydrate substituents in 5-position (Figure 96).

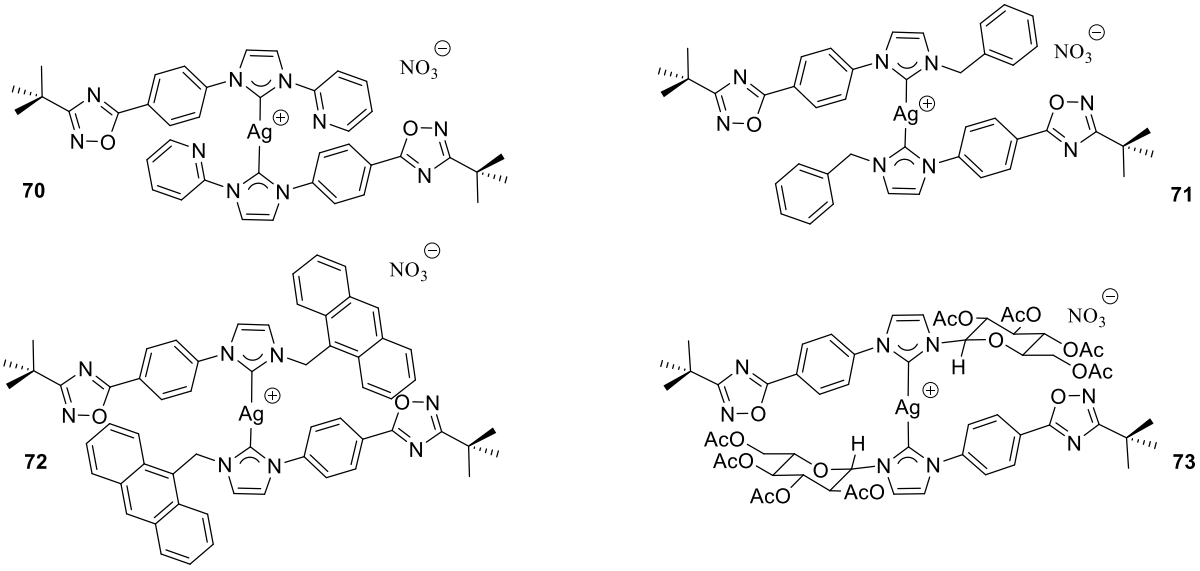
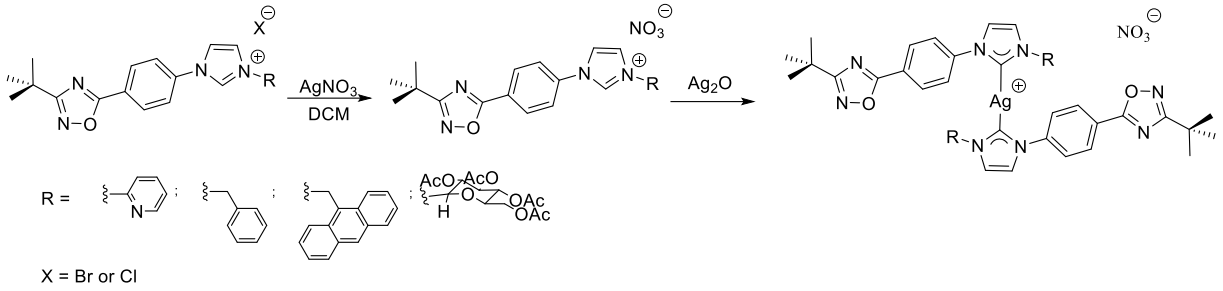


Figure 96. Target cationic Ag(I)-NHC complexes.

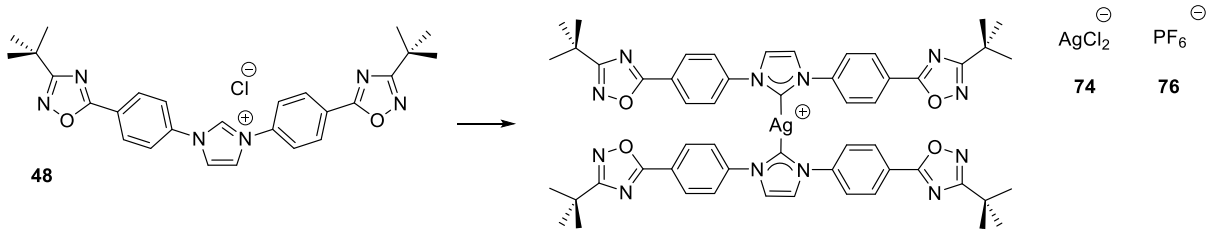
These complexes were synthesized by reacting the analogous imidazolium nitrate salts with Ag_2O. As shown in Scheme 74 the anion exchange from the imidazolium bromide or chloride salts was performed by treatment with an equivalent amount of $AgNO_3$ in dry DCM. The imidazolium nitrate salts were isolated and immediately reacted with two molar equivalents of Ag_2O in order to afford the target silver complexes **70-73** in moderate yields (47-67%).



Scheme 74. Synthesis of Ag(I)-NHC nitrate complexes.

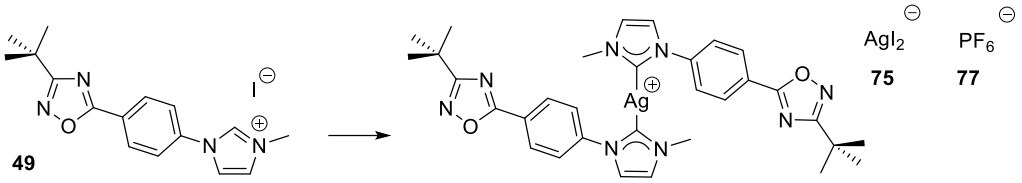
The generation of the new Ag(I)-NHC complexes **70-73** was confirmed by the disappearance of the acidic proton signals of the imidazolium salts (at 10.88, 10.41, 10.49 and 11.09 ppm, respectively). Upon complexation with silver ions, the ^{13}C NMR resonances of the carbene C-atom is detected in the range of 178.43-180.58 ppm (178.43, 179.03, 180.58, 179.01, ppm). Furthermore, positive-ion mode ESI mass spectra of these complexes are dominated by [M^+ - NO_3] fragment peaks arising from the loss of the NO_3 anion.

The Ag(I) intermediate complexes generated during the salt metathesis reaction (**74-77**) were also characterized and tested for their antitumor activity. The synthetic route is presented in schemes 75 and 76.



Scheme 75. Synthesis of complex Ag(I)-transmetallation intermediates **74** and **76**.

For the synthesis of Ag(I)-NHC complexes **74** and **75** the basic silver precursors (silver oxide) was used to deprotonate the imidazolium salts *in situ*. Reaction of 1,3-bis(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-imidazolium chloride (**48**) and 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-methyl-imidazolium iodide (**49**) with an stoichiometric amount of silver oxide in CH_3CN at 60°C, produced the corresponding Ag(I)-NHC complexes **74** and **75**, respectively. The expected signals were observed in the ^1H and ^{13}C NMR spectra. The MS-ESI spectra show the cationic fragment peaks [M^+ - $AgCl_2$ respective M^+ - AgI_2]. In order to confer a better solubility to the Ag(I)-NHC complexes, the negative counterions $[AgCl_2]^-$ and $[AgI_2]^-$ were exchanged by the $[PF_6]^-$ anion. Compounds **76** and **77** were also tested for their antitumor activity.



Scheme 76. Synthesis of complex Ag(I)-transmetallation intermediates **75** and **77**.

IV.4.3. *In vitro* anti-tumor activity of silver(I)-NHC complexes with 1,2,4-oxadiazole substituents towards human tumor cell lines.

Compounds **68-77** were tested *in vitro* for anti-tumor activity in a panel of 11 human tumor cell lines by using a monolayer cell survival and proliferation assay. As shown in Figure 97, very good potency with mean IC_{50} values <0.3 µM was detected for 2 compounds, namely **73** (mean IC_{50} = 0.19 µM and **70** (0.22 µM). Additionally, good activity was detected also for **71** (mean IC_{50} = 0.53 µM) and at a lower level for **72** (mean IC_{50} = 1.8 µM). Moreover, compound **75** presented moderate activity with a mean IC_{50} value of 17.18 µM and, at a lower level, anticancer activity was found for **74**, **76**, **77**, **68** and **69** with mean IC_{50} values in the range from 31.21 µM to 58.5 µM.

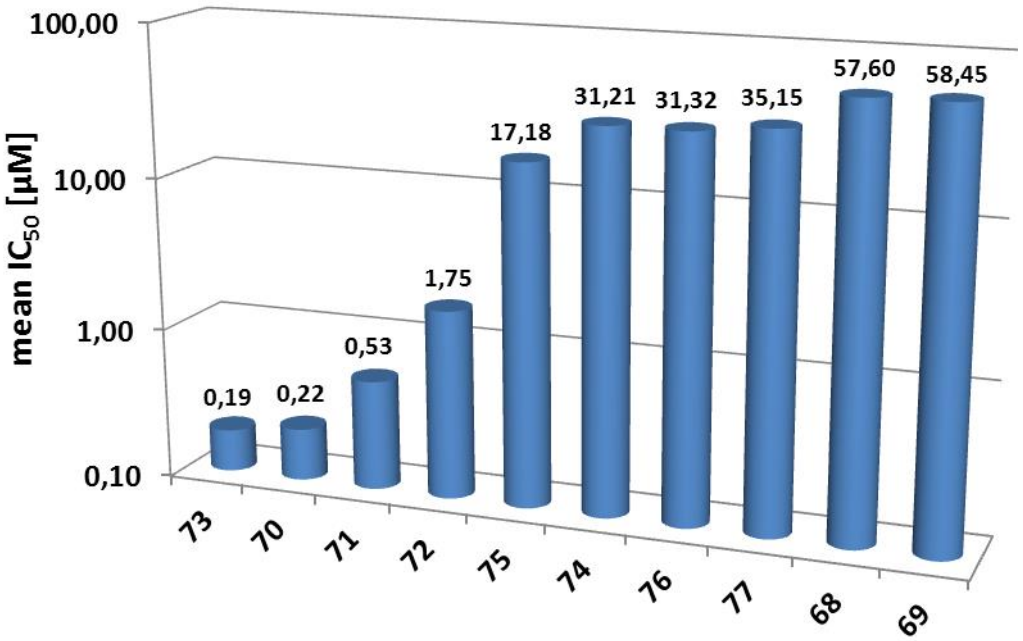


Figure 97. In vitro anti-tumor activity of compounds **68-77** in a panel of 12 human tumor cell lines (mean IC_{50} values).

As illustrated in the IC_{50} heatmap presentation (Figure 98), the two most active compounds reveal a selective activity profile across the 12 cell lines tested, with the cell lines UXF 1138 (cancer of the uteri body), LXFA 629 (lung cancer), MAXF 401 (mammary

cancer) and MEXF 462(melanoma) presenting above-average sensitivity. In the same time, the resistant cell lines are OVXF 899 (ovarian cancer) and RXF 486 (renal cancer).

		Cell lines											
Compound	Unit	CXF HT-29	GXF 251	LXFA 629	LXFL 529	MAXF 401	MEXF 462	OVXF 899	PAXF 1657	PXF 1752	RXF 486	UXF 1138	geomean IC50 [µM]
73	µM	0,33	0,22	0,13	0,08	0,07	0,05	2,48	0,07	0,24	4,13	0,03	**0,19**
70	µM	0,56	0,06	0,93	0,08	0,05	0,08	0,36	0,40	0,10	1,26	0,30	**0,22**
71	µM	0,89	0,22	2,82	0,17	0,19	0,22	0,73	0,80	0,22	2,64	0,77	**0,53**
72	µM	3,42	0,73	2,17	0,95	1,12	2,23	7,64	2,16	0,81	3,91	0,72	**1,75**
75	µM	18,06	9,73	32,66	18,33	10,77	28,10	29,37	10,69	16,38	12,78	18,44	**17,18**
74	µM	40,11	30,00	45,69	22,13	14,27	31,58	100,00	39,89	22,83	40,90	13,38	**31,21**
76	µM	47,90	19,37	100,00	27,23	10,65	28,01	100,00	37,67	20,05	46,63	10,71	**31,32**
77	µM	44,22	17,46	83,48	36,73	12,67	53,12	56,84	19,30	31,68	49,70	36,77	**35,15**
68	µM	85,10	39,35	100,00	49,85	20,92	95,29	100,00	53,31	40,02	100,00	32,66	**57,61**
69	µM	78,84	33,98	100,00	41,29	36,41	100,00	100,00	43,16	42,75	100,00	36,62	**58,45**

1/32 1/16 1/8 1/4 1/2 1 2 4 8 16 32 -fold mean IC_{50}

sensitive cell lines — resistant cell lines

Figure 98. Heatmap presentation of individual IC_{50} values for compounds **68**-**77** in a panel of 11 human tumor cell lines.

From the transmetallation intermediates, compound **75**, displayed individual IC_{50} values in the range from 9.73 µM (GXF 251) to 32.66 µM (LXFA 629), but showed no pronounced selectivity for any of the tested cell lines. The other compounds showed moderate selectivity towards MAXF 401 (breast cancer).

For comparison five platinum compounds of the first and second generation were tested and they exhibited geometric mean IC_{50} values across the 12 cell line panel in the range from 0.96 µM (Satraplatin) to 11.68 µM (Carboplatin).

		Cell lines											Geom. mean IC_{50} [µM]
compound	unit	CXF HT-29	GXF 251	LXFA 629	LXFL 529	MAXF 401	MEXF 462	OVXF 899	PAXF 1657	PRXF 22Rv1	PXF 1752	RXF 486	UXF 1138	
Satraplatin	µM	2,07	1,11	1,32	0,82	0,27	0,75	0,57	1,11	0,46	3,07	2,83	0,47	**0,96**
Tetraplatin	µM	1,29	0,43	18,27	0,84	0,40	2,33	0,46	2,69	0,70	8,10	1,05	2,11	**1,49**
Oxaliplatin	µM	1,28	1,55	12,13	3,37	0,60	5,32	0,21	4,77	0,71	15,34	0,69	3,36	**2,08**
Cisplatin	µM	14,55	4,97	4,71	0,83	0,68	0,73	2,54	7,62	3,57	38,58	8,79	2,77	**3,80**
Carboplatin	µM	26,16	9,19	19,65	4,69	1,98	4,04	8,01	36,61	9,99	52,31	27,68	8,53	**11,68**

1/32 1/16 1/8 1/4 1/2 1 2 4 8 16 32 -fold mean IC_{50}

sensitive cell lines — resistant cell lines

Figure 99. Heatmap presentation of individual IC_{50} values for approved Platinum compounds.

Specifically **73, 70,** and **71** were undoubtedly more potent than the platinum reference compounds. Complex **72** proved to be the less active from the series, but still with a potency similar to the platinum derivatives Tetraplatin (IC_{50} = 1.49 µM) and Oxaliplatin (IC_{50} = 2.08 µM). The other compounds **68**-**77** are outside the activity range of the platinum drugs.

IV.5. Synthesis and structural characterization of gold(I) bis-NHC complexes

IV.5.1. General Aspects

Baker and co-workers were one of the first research groups that presented the synthesis and biological evaluation of linear, two-coordinate homoleptic cationic Au(I)-NHC complexes as mitochondrial antitumor agents. In order to improve the lipophilicity of the compounds, they inserted several alkyl substituents on the imidazolium ring, maintaining the main core of the complex unchanged.[366]

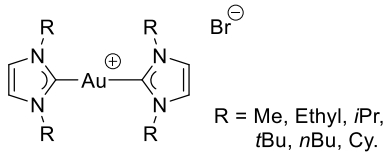


Figure 100. Cationic NHC-Au(I) complexes reported by Baker *et al.*.

Having as main target selective apoptosis in tumor cell lines, Weaver *et al.* develop besides the neutral gold(I) NHC complexes, a series of cationic gold(I) NHC complexes.[90] Although these complexes proved to be more active than the corresponding neutral derivatives, no selectivity was observed.

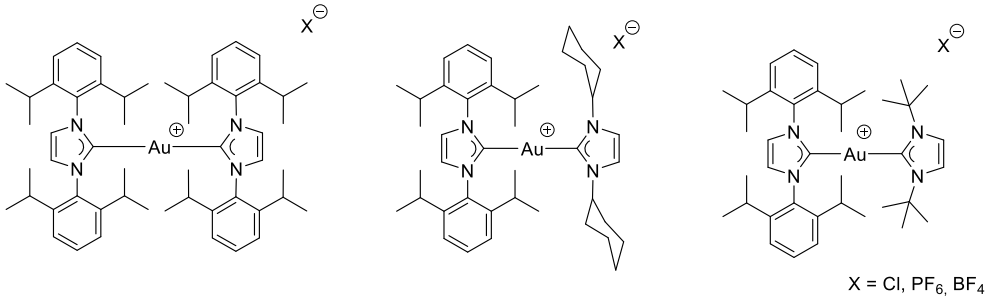


Figure 101. Cationic NHC-Au(I) complexes reported by Weaver *et al.*.

Comparable cationic NHC-Au(I)-complexes having 1,3-benzimidazol-2-ylidene core were evaluated as thioredoxin reductase (TrxR) inhibitors as well as for their antimitochondrial properties by Rubbiani *et al.*.[91] The positive charged complexes showed remarkable antiproliferative effects against MCF-7 (human breast adenocarcinoma), HT-29 (colon adenocarcinoma) and HEK-293 cells (human embryonic kidney), with IC_{50} values in the micromolar and submicromolar area.

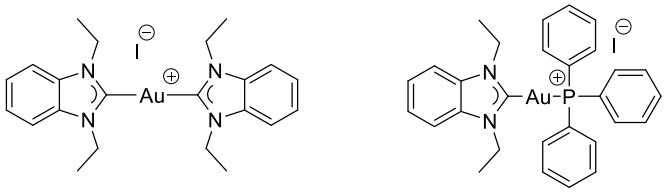


Figure 102. Cationic NHC-Au(I) complexes reported by Rubbiani *et al.*.

Gust *et al.* also prepared NHC-Au(I)-complexes in analogy to his neutral compounds.[94]

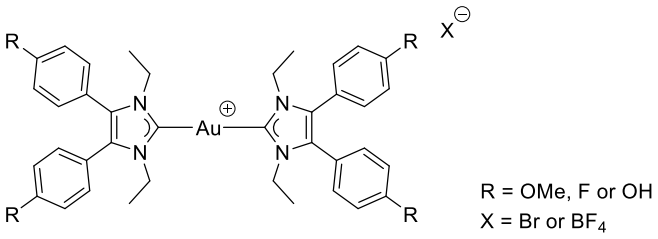


Figure 103. Cationic NHC-Au(I) complexes reported by Gust *et al.*.

He compared the growth inhibitory activity of the bis-NHC cationic complexes with the activity of the neutral NHC gold complexes and observed a boost of the activity in the case of breast and colon cancer cell line. A more exotic example is the caffeine related NHC gold(I) charged complexes developed by Casini *et al.*.[367] These complexes were investigated *in vitro* against human lung cancer and human ovarian cancer cell lines. All compounds showed relative moderate activities.

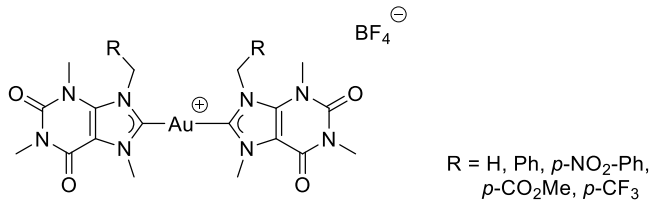


Figure 104. Cationic NHC-Au(I) complexes reported by Casini *et al.*.

IV.5.2. Synthesis and structural characterization

A series of five novel 1,2,4-oxadiazole related gold(I) bis-NHC complexes with various ligands were designed, isolated, characterized and tested for anti-tumor activity. The molecular structures of the new synthesized compounds are showed in Fig. 105.

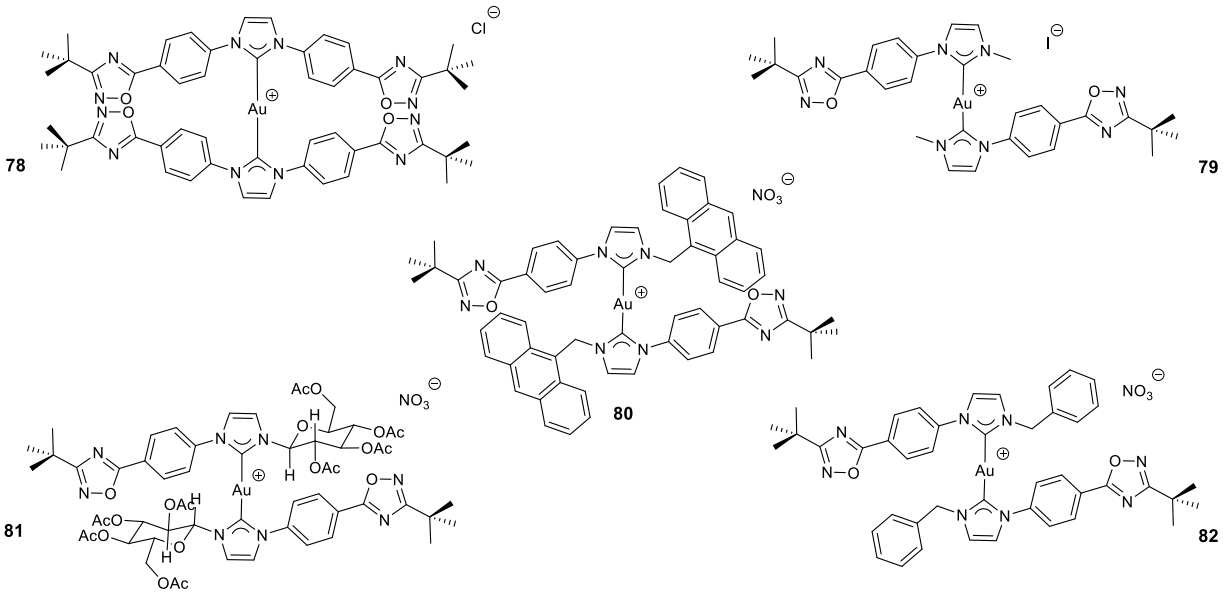
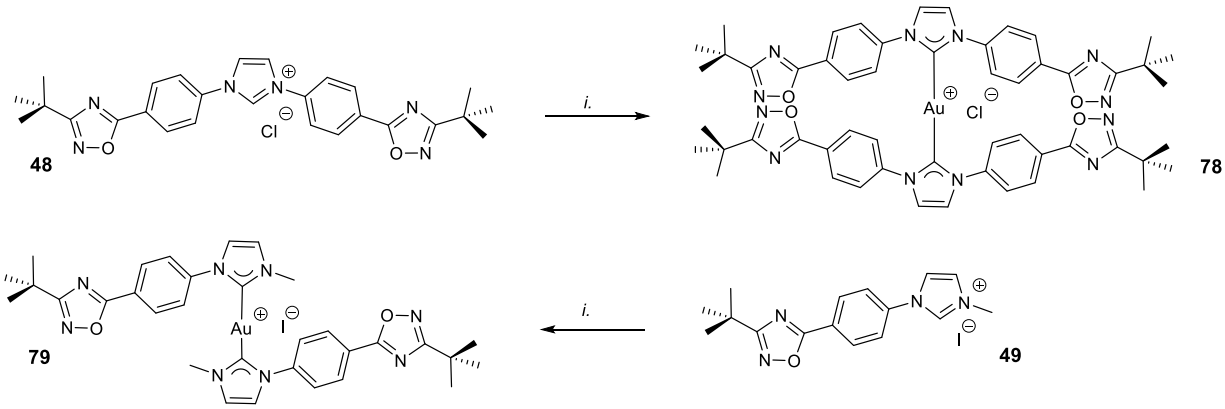


Figure 105. Synthesized gold(I) bis-NHC complexes (**78** - **82**).

IV.5.2.1. Synthesis starting from the imidazolium salts

The gold(I) bis-NHC complexes **78** and **79** were prepared directly from the correspondent imidazolium salts **48** respectively **49** as shown in Scheme 77. The first trying for building-up these two complexes involved a two-step reaction. First one can generate the Ag(I)-NHC cationic complex (**74** and **75**) and then replace the silver atom with gold. The reaction worked but the isolated yield was less than 15%. In order to produce a better yield other bases, solvents and reaction conditions were screened. The best isolated yields for these two complexes were obtained when K_2CO_3 was used for the deprotonation in acetone at reflux (56% respectively 43%).



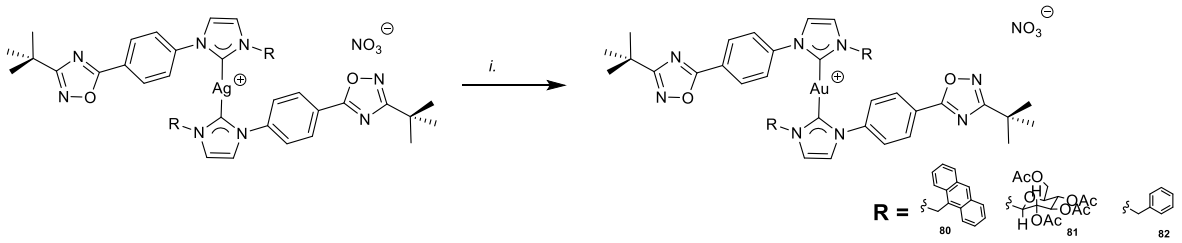
Scheme 77. Synthetic route for gold(I) bis-NHC complexes **78** and **79**. Reagents and conditions: *i*. K_2CO_3, $AuCl(SMe_2)$, acetone, 60 °C, 24 h.

The two complexes were isolated and purified by flash column chromatography and characterized by IR, ^{1}H NMR, ^{13}C NMR spectroscopy and HR-ESI spectrometry. The acidic proton signals in the ^{1}H NMR spectrum disappears and a strong shift of NCN-Au resonance in

the ^{13}C NMR was observed for both compounds (184.3 and 182.8 ppm). The high resolution electrospray ionization mass analyses sustained the NMR interpretation. The cationic core of the two complexes showed the entire isotopic pattern.

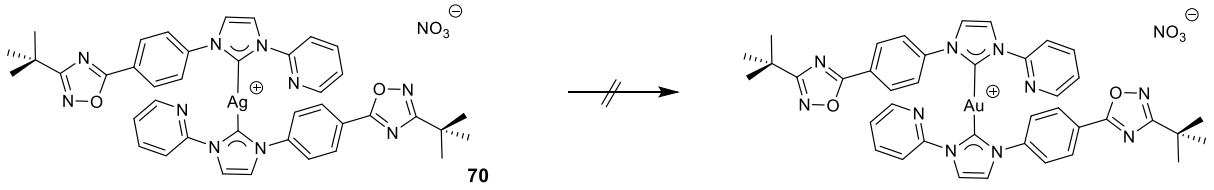
IV.5.2.2. Synthesis of gold(I) bis-NHC complexes starting from the Ag(I)-NHC analogues

Other gold(I) bis-NHC complexes of 1,2,4-oxadiazole-2-ylidene class (**80**, **81** and **82**) with diverse bioactive ligands (9-methyl-antracene, 2,3,4,5-tetra-*O*-acetyl-*D*-glucopyranosyl and benzyl) were also designed, structurally characterized and biologically investigated for antitumor activity.



Scheme 78. Synthetic route for gold(I) bis-NHC nitrate complexes **80** - **82**. Reagents and conditions: *i*. AuCl(SMe_2), DCM, rt, 12 h.

The synthesis of these complexes was accomplished using the transmetallation route via their silver(I) bis-NHC analogues **71**, **72** and**73**. The Ag(I)-NHC derivatives were reacted with AuCl(SMe_2) in an 1:1 ratio in dry DCM in the absence of light. However, any attempt to obtain gold(I) bis-NHC complexes of 1,2,4-oxadiazole-2-ylidene complex with 2-pyridine ligand starting from the silver complex **70** failed.



Scheme 79. Unsuccessful transmetallation reaction.

The reaction was repeated several times with small modifications in the synthetic protocol (lower temperatures, shorter reaction time), but in all cases an unidentified pink solid formed.

The new gold(I) complexes with the $[NO_3]^-$ counterion **80-82** were purified by flash column chromatography and characterized by IR, ^1H NMR, ^{13}C NMR spectroscopy and HR-ESI spectrometry. The carbene carbon resonances were found at 183.84, 180.87 and 182.39 ppm, respectively, similar to the resonances of their silver analogs, 180.59, 179.01 and 179.03

ppm, respectively. The high resolution ESI mass spectrometry confirmed beyond any doubt the proposed molecular composition.

IV.5.3. *In vitro* anti-tumor activity towards human tumor cell lines.

The *in vitro* anti-tumor activity of the compounds **78-82** was assessed in a panel of 11 human tumor cell lines by using a monolayer cell survival and proliferation assay. In Figure 106 shows the mean IC_{50} values of the tested compounds. The carbohydrate containing complex **81**, with a mean IC_{50} = 0.8 µM is the most potent candidate from this series. Good potency was also registered for **82** and **78** with a mean IC_{50} of 1.41, respectively 1.87 µM. the other two complexes showed week anti-proliferation properties.

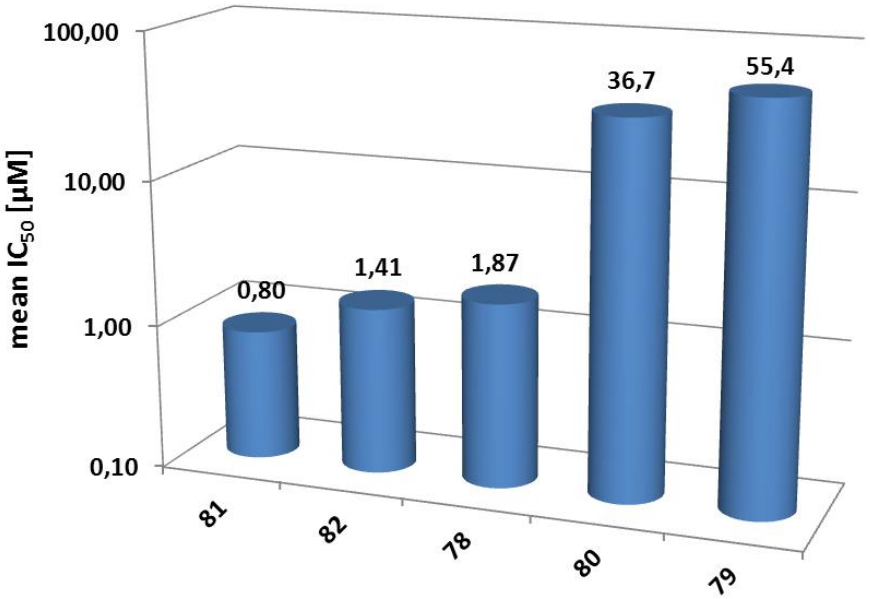


Figure 106. In vitro anti-tumor activity of compounds **78-82** in a panel of 11 human tumor cell lines (mean IC_{50} values).

Figure 107 shows the individual IC_{50} values as a heatmap presentation. The most effective compounds, **81**, **82** and **78**, displayed individual IC_{50} values in the range from 0.12 µM (GFX 251) to 35.51 µM (OVXF 899). The three complexes were selective for GXF 251, MAXF 401 and PXF 1752. The other two tested compounds **79** and **80**, showed no pronounced selectivity for any of the cell lines investigated.

		Cell lines											
Compound	Unit	CXF HT-29	GXF 251	LXFA 629	LXFL 529	MAXF 401	MEXF 462	OVXF 899	PAXF 1657	PXF 1752	RXF 486	UXF 1138	geomean IC50 [μM]
81	μM	0,70	0,12	0,49	0,56	0,38	0,78	10,20	0,61	0,40	12,60	0,43	**0,80**
82	μM	1,77	0,65	1,33	1,16	0,56	0,82	3,82	1,80	0,94	8,75	0,96	**1,41**
78	μM	1,66	1,05	1,85	1,22	0,52	1,36	35,51	0,92	0,88	22,45	0,55	**1,87**
80	μM	40,50	21,70	48,40	42,90	31,10	20,50	100,00	54,00	25,40	100,00	10,10	**36,67**
79	μM	100,00	23,40	21,30	100,00	23,70	46,40	100,00	100,00	66,60	100,00	41,40	**55,41**

1/32 1/16 1/8 1/4 1/2 1 2 4 8 16 32 -fold mean IC_{50}

sensitive cell lines — resistant cell lines

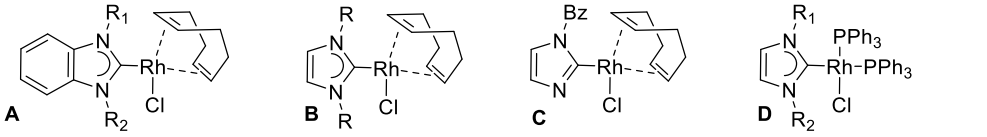
Figure 107. Heatmap presentation of individual IC_{50} values for compounds **78-82** in a panel of 11 human tumor cell lines.

IV.6. Synthesis and structural characterization of Rhodium(I) NHC complexes

IV.6.1. General Aspects

Apart from the well-known gold and silver, other metals like ruthenium[368,369] or rhodium[370,371] have also been employed as bioactive cores of NHC complexes.[85,372]

NHC complexes with rhodium and ruthenium were one of the first compounds described as antibacterial agents against *Enterococcus faecalis*, *P. aeruginosa*, *E. coli* and *S. aureus*. In this area of medicinal chemistry Cetinkaya *et al*. investigated a large library of rhodium and ruthenium NHC derivatives (Figure 108).[370]



R_1 = Me, Et
R_2 = Me, Et

R = Me, Bz, p-tolyl, p-methoxyphenyl, methoxyethyl

Figure 108. Examples of bioactive rhodium(I)-NHC derivatives.

The best results, very active and selective in the inhibition of Gram-positive bacteria strains, were registered by the complexes in which R_1 and R_2 are methyl and ethyl groups, respectively. The ruthenium analogues proved to be less active as antibacterial agents. Aside from antibacterial activity, rhodium complexes have been widely studied as potential anticancer agents, especially the ones which contain Rh(III) coordinated to various ligands (polypyridyls or cyclopentadienyls).[80,92,95,373-378] The Rh(I) derivatives are not very often mentioned in biological research studies for their antitumor activity[93,94,371,379,380]. One of the few examples is the work of McAlpine *et al*.,[371] who reported Rh(I)-NHC derivatives that can modify the cell migration, DNA replication and DNA condensation.

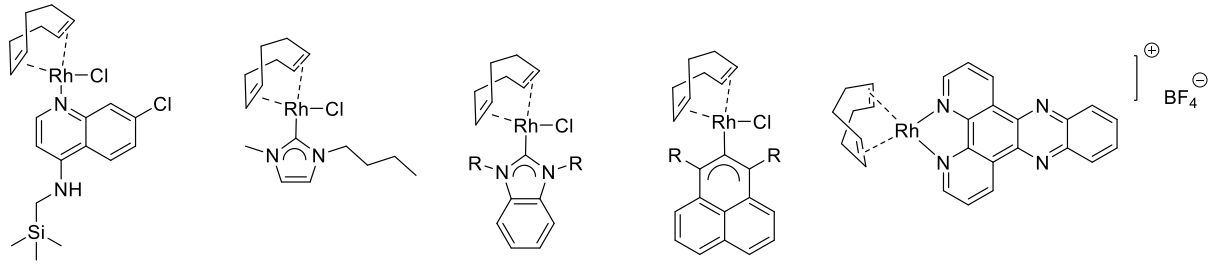
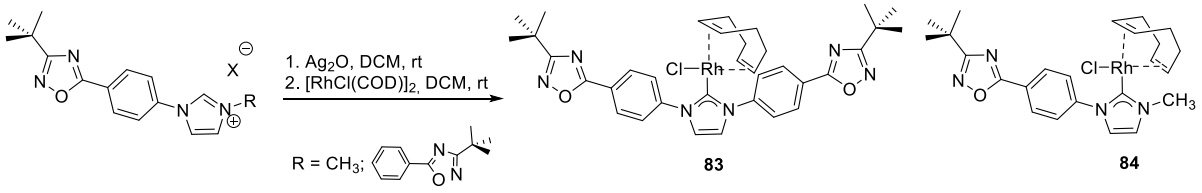


Figure 109. Examples of rhodium complexes with antitumor activity.

The fact that Rh(I) is isoelectronic with Pt(II) and that Rh(I) complexes displays a square-planar geometry similar to already marketed cisplatin derivatives, makes the Rh(I) a good metal center for possible antitumor activity. Additionally, the new Rh(I) derivatives possess the NHC core bearing 1,2,4-oxadiazole moiety and the COD ligand coordinating to the Rh(I) center. These moieties possess physico-chemical properties that could influence the biological activity of the target molecules.

IV.6.2. Synthesis and structural characterization of Rh(I)-NHC complexes

The most common synthetic route to generate Rh(I)-NHC complexes consist in the deprotonation of the imidazolium salt precursor. This can be achieved easily by using a strong base[381] or a basic ligand.[238] The employment of the free carbene is somehow limited by the dry, air-free conditions in which the reaction must be performed. At the same time the functionalities attached to the ligand are playing an important role. The path chosen involved two steps as shown in Scheme 80.



Scheme 80. Synthetic route for Rh(I)-NHC complexes bearing 1,2,4-oxadiazol moiety.

The rhodium-NHC complexes, Chloro(η^4-1,5-cyclooctadiene)-(1,3-bis(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-1*H*-imidazol-2(3*H*) ylidene)-rhodium(I) (**83**) and Chloro(η^4-1,5-cyclooctadiene)-(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-methyl-1*H*-imidazol-2(3*H*)-ylidene)rhodium(I) (**84**), were prepared in moderate yields (59% and 44% respectively) using the transmetallation path. The $[RhCl(COD)]_2$ was treated with the corresponding silver(I)-carbene complexes according to already established procedures.[248,382-386] In the first step it was generated Ag(I)-NHC complexes as intermediates. This was prepared *in situ* starting from the 1,2,4-oxadiazole-containing imidazolium salts (**48**-**49**) and silver oxide in

DCM at room temperature. The clear solution of Ag(I)-NHC complexes in DCM was slowly added (or added dropwise) into a solution of $[RhCl(COD)]_2$ in DCM to afford the desired metal complex after purification using column chromatography. The isolated complexes are air and moisture stable.

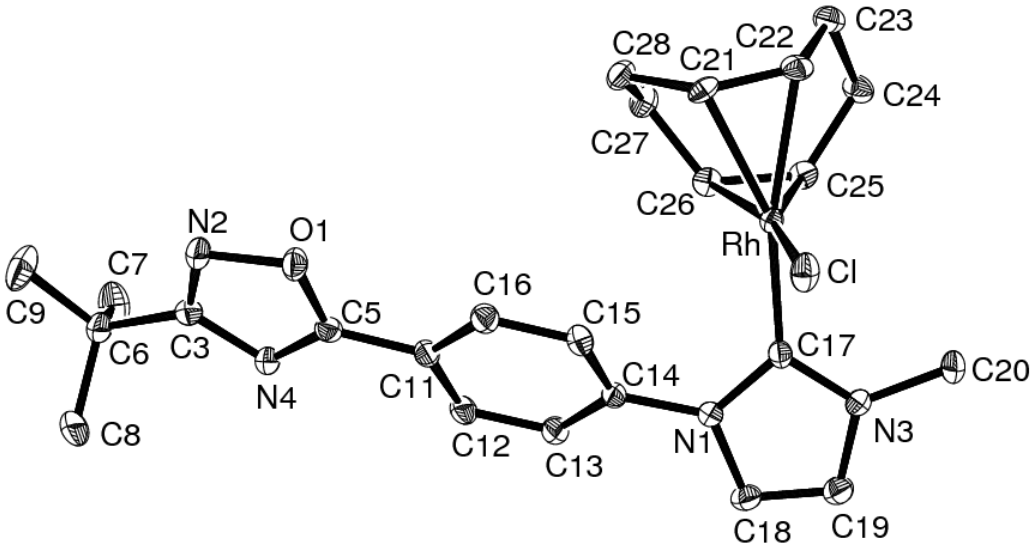


Figure 110. ORTEP representation of Chloro(η^4-1,5-cyclooctadiene)-(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-methyl-1*H*-imidazol-2(3*H*)-ylidene)rhodium(I) (**84**) with thermal ellipsoids drawn at the 50% probability level. Hydrogen and disordered atoms were omitted for clarity. Selected bond lengths (Å) and angles (°):Rh-C(17) 2.021(2), Rh-Cl 2.3860(5), Rh-C(21) 2.1941(19), Rh-C(22) 2.2284(18), Rh-C(25) 2.113(2), Rh-C(26) 2.1230(18), C(17)-Rh-Cl 88.58(5), N(3)-C(17)-N(1) 104.39(17), C(17)-N(3)-C(20) 123.90(17), C(17)-N(1)-C(14)-C(15) -35.2(3).

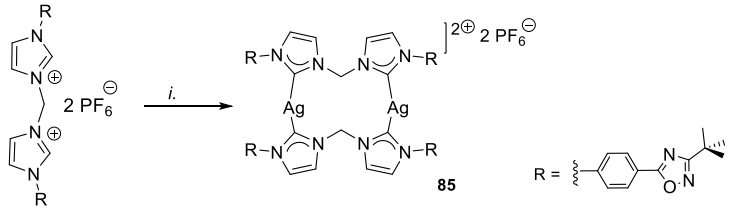
The two Rh(I) complexes **83** and **84** were characterized as monomeric species by IR, ^{1}H NMR, ^{13}C NMR spectroscopy, mass spectrometry and elemental analyses. For **83** the ^{1}H NMR spectra, aside from the COD specific signals, shows two multiplets corresponding for the eight aromatic protons at 8.60 and 8.36 ppm, one singlet at 7.44 ppm for the two protons of the imidazole ring and one singlet for the two *t*-butyl groups at 1.45 ppm. The ^{1}H NMR spectra of complex **84** revealed the four aromatic protons as multiplets at 8.40 and 8.33 ppm, two doublets at 7.22 and 7.04 ppm for the imidazole ring protons with the J_{HH} = 1.9 Hz, one singlet at 4.24 ppm for the methyl protons and nine protons at 1.46 ppm for *t*-butyl. The ^{13}C NMR doublets for the carbenic carbons appear at δ = 185.9 (J_{RhC} = 52.4 Hz) for **83** and 184.35 (J_{RhC} = 51.29 Hz) for **84**. The positive ion MS-ESI analysis for complexes **84** showed as major peak the *m/z* $[M\text{-}Cl]^+$ (493.1) fragment along with *m/z* $[M + H]^+$ (528.1). The *m/z* EI analysis for **83** shows the molecular peak M^+ (714.2) along with fragments resulted from the loose of Cl and COD moieties. Crystals suitable for X-ray crystallographic analysis were obtained by slow evaporation of a concentrated solution of **84** in DCM and thereby prove the structure of the complex.

As depicted in Figure 110 the central rhodium atom is coordinated by the 1,3-cyclooctadiene unit, the chlorine atom and the corresponding carbene ligand in a typical square planar coordination. The bond length of Rh-$C_{carbene}$, Rh-Cl, Rh-C_{COD} and $C_{carbene}$-N are in the range observed for other Rh(I)COD-NHC complexes.

IV.7. Synthesis and structural characterization 1,2,4-oxadiazole bridged bis-NHC complexes

IV.7.1. Synthesis of Ag(I) bis-NHC complex (85)

The original poiect-plan involved the synthesis of some binuclear gold 1,2,4-oxadiazol derivatives[387,388] and test their antitumor activity. In an attempt to generate the binuclear gold cationic complex **67**, we first attempted to synthesize the binuclear silver cationic complex **85** by the same method but using acetonitrile as reaction solvent.[388] The silver complex was obtained and characterized, but all attempts to isolate the gold derivative were unsuccessful.



Scheme 81. Synthesis of binuclear Ag(I) 1,2,4-oxadiazole; *i.* Ag_2O, [Au(DMS)Cl], DCM, rt.

X-ray crystallographic analysis of derivative **85** confirms the presence of a dinuclear, dicationic complex with two dicarbene ligands, each of which bridges both silver atoms. (Fig. 111). Similar dimeric structures have been observed for silver complexes of chelating CNC pincer[389,390] and C_2N_2 cyclophane bis(NHC) ligands.[391] The Ag-C bond lengths (Ag1-C1 2.071(5), Ag1-C3 2.090(5), Ag2-C2 2.087(5), Ag2-C4 2.085(5) Å) and near-linear C-Ag-C angles (169.85(17), 167.28(16)°) of **85** lie within the normal ranges for published silver NHC complexes.

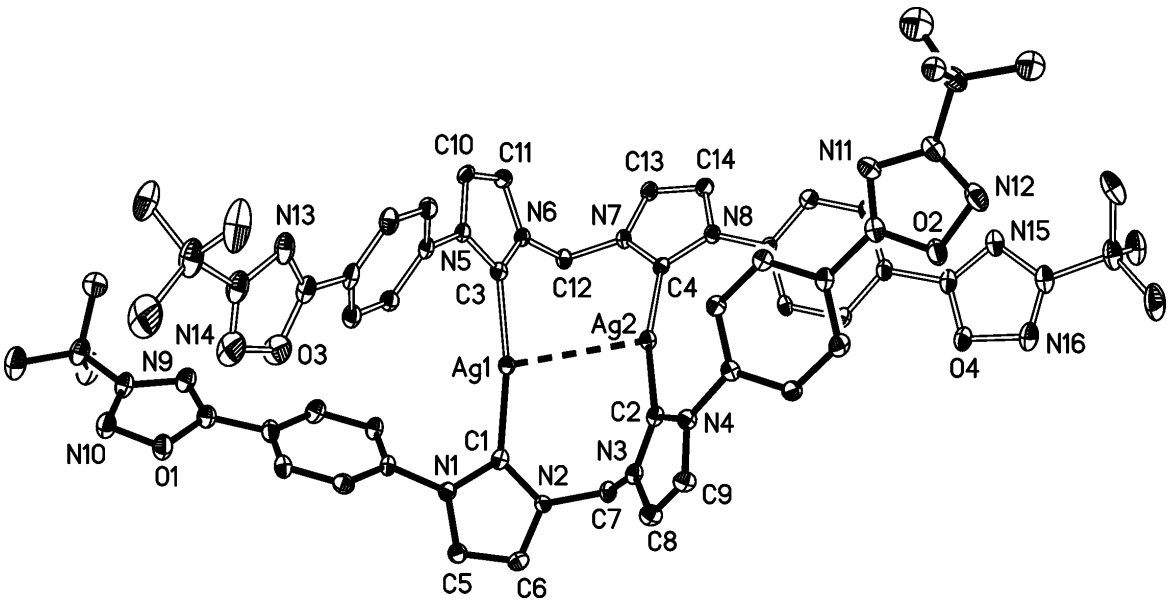
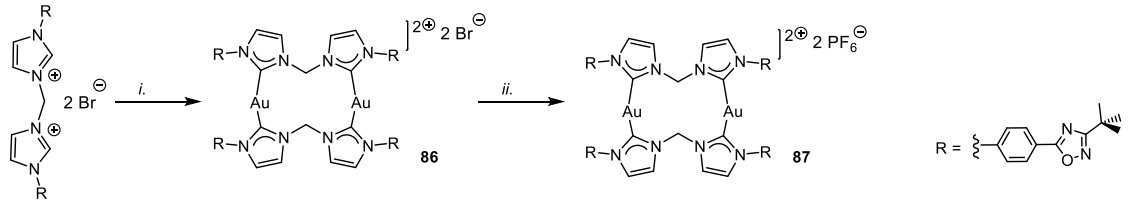


Figure 111. Structure of the dicationic silver complex **85**. Atoms are drawn as 30% thermal ellipsoids. Hydrogen atoms are omitted for clarity. Only one position of the disordered group (to the right of N4 in the lower ligand) is shown.

An important characteristic of **85** is the short AgΛAg contact of 3.1642(4) Å. Such contacts shorter than twice the van der Waals radius (3.40 Å) are considered diagnostic for "argentophilic" interactions,[392] but these are not as common as their AuΛAu counterparts.

IV.7.2. Synthesis of Au(I) bis-NHC complexes (86 and 87)

In order to produce the compound **86**, it was necessary to heat the imidazolium salt in DMF at high temperature in the presence of NaOAc as base. The yield after purification was moderate (40%). In order to increase the solubility of the di gold complex in organic solvents, it was necessary to change the counterion from $[Br]^-$ to $[PF_6]^-$.

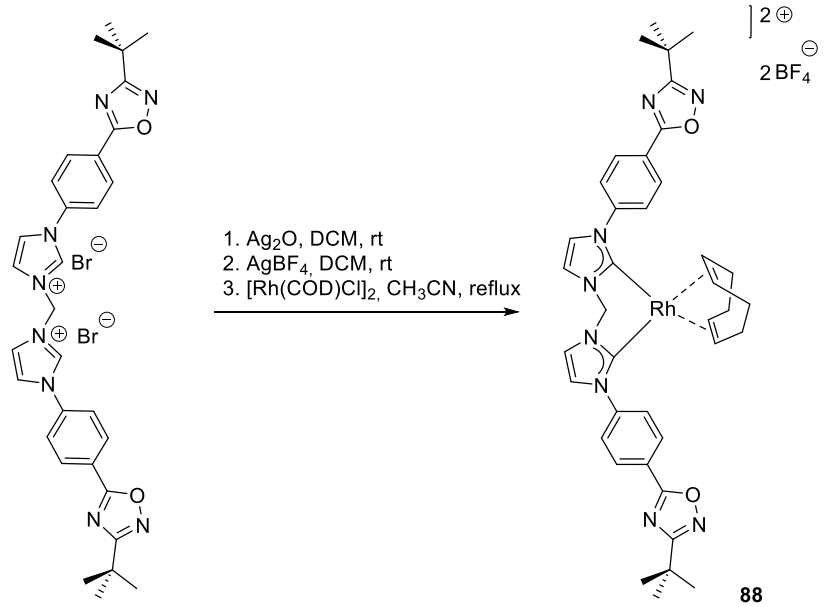


Scheme 82. Synthesis of binuclear Au(I) 1,2,4-oxadiazole; *i.* NaOAc, [Au(DMS)Cl], DMF, 100°C; *iii*. NH_4PF_6, H_2O, rt.

The structures of the new binuclear gold compounds were confirmed by IR, NMR spectroscopy and hight resolution MS-ESI. Evenmore the elemental analyses were in accordance with the calculated ones.

IV.7.3. Synthesis of Rh bis-NHC complex (88)

The {1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylene-diimidazolin-2,2′-diylidene}Rh(η^2:η^2-COD)[BF_4] (**88**) was prepared in accordance with literature.[433] Starting from 1,1′-[5-(phenyl)-3-(*tert* butyl)-1,2,4-oxadiazole]-3,3′-methylenediimidazolium bis(bromide) (**57**), it was possible to produce the bis-NHC-Ag(I) intermediate in a short time by adding a slight excess of Ag_2O in DCM at room temperature. The brown solid thus obtained after filtration through Celite and removal of the solvent was used further as carbine transfer reagent without any further purification. In a second step the silver derivative was treated with $AgBF_4$ in acetonitrile at reflux. Precipitation from DCM upon addition of ethanol afforded the biscarbene complex **88** as pure compound (Scheme 83).

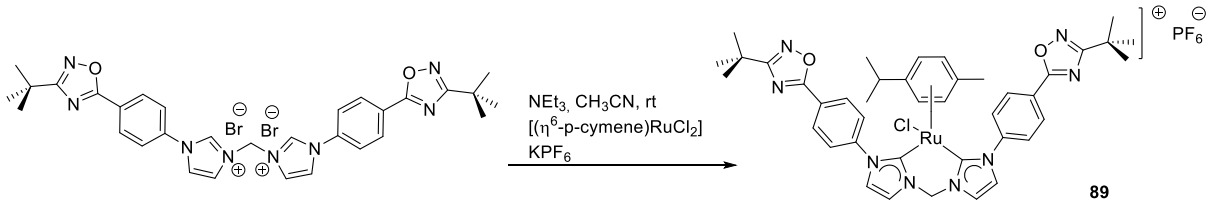


Scheme 83. Synthetic route for {1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylene-diimidazolin-2,2′-diylidene}Rh(η^2:η^2-COD)[BF_4].

The Rh(I)-bridged biscarbene **88** indicates the ^{1}H NMR spectroscopy the expected signals for COD moiety along with two multiplets corresponding for the eight aromatic protons at 8.40 and 7.95 ppm, two doublets at 7.89 and 7.29 ppm related to the four protons of the imidazole rings with the J_{HH} = 1.98 Hz, and one singlet for the two *t*-butyl groups at 1.49 ppm. The two hydrogen atoms of the CH_2 linker are geometrically inequivalent, giving in the ^{1}H NMR spectrum two separate doublets (J_{HH} = 12.9 Hz). The ^{13}C NMR doublet for the carbenic carbons appear at δ = 179.5 (J_{RhC} = 53 Hz). The positive ion MS (HR-ESI) analysis for complex **88** illustrate as main signal the *m/z* [M^{2+} (M-2xBF_4^-)] (759.2642) and the *m/z* [M^{2+}-COD] (651.1701) as a fragment.

IV.7.4. Synthesis of Ru bis-NHC complex (89)

The {1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylene-diimidazolin-2,2′-diylidene}RuCl(η^6-*p*-cymene)[PF_6] (**89**) was synthesized using a modified published protocol.[393] Reaction of the imidazolium salt precursor 1,1′-[5-(phenyl)-3-(*tert* butyl)-1,2,4-oxadiazole]-3,3′-methylenediimidazolium bis(bromide) with [(η^6-*p*-cymene)$RuCl_2$]$_2$ resulted in the expected bis-carbene complex in low yield (23%). The reaction mixture was treated with NH_4PF_6 in order to obtain the final cationic complex as $[PF_6]^-$ salt (Scheme 84).

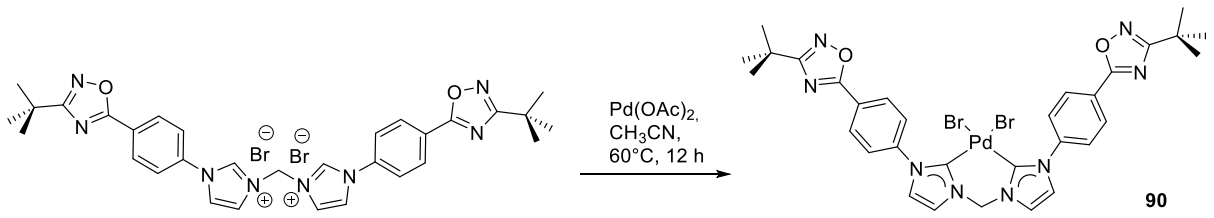


Scheme 84. Synthetic route for {1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylene-diimidazolin-2,2′-diylidene}RhCl(η^6-*p*-cymene)[PF_6] (**89**).

The complex was characterized by IR, NMR spectroscopy and mass spectrometry. The two imidazole rings are equivalent according to ^{1}H NMR spectrum, confirming the 2-fold symmetry of the chelatíng ligand. The two hydrogen atoms of the methylene bridge are also inequivalent like in the case of the bis-carbene Rh(I) complex **89**. The two hydrogen atoms shows two separate doublets with J_{HH} = 13.1 Hz. The carbene carbon atom was found in the ^{13}C NMR spectra in the usual high frequency area (δ = 176.53 ppm). Positive ion MS (HR-ESI) analyses of the isolated Ru(I) derivative showed a peak at *m/z* [M^+] (819.2479).

IV.7.5. Synthesis of Pd bis-NHC complex (90)

The optimal reaction conditions for the synthesis and isolation of palladium bis-carbene complex **90** is pictured in Scheme 85.



Scheme 85. Synthetic aproach for compound **90**.

Initial atempts to generate complex **90** using published procedures,[394,395] where the reaction was performed in THF or DMSO, were unsatisfactory. Due to the low yield (lower than 15%) and the decomposition of the compounds involved in the reaction, the solvent and

the temperature of the reaction were changed. In order to isolate complex **92** in pure form and good yield (67%), it was nesessary to use acetonitril as solvent at 60°C for a period of 12 hours.

The palladium complex was fully characterized. The ^{1}H NMR resonances are broader than those in the corresponding Rh or Ru biscarbene derivatives. The main difference appears at 8.95 ppm, where the protons from the two imidazole rings are more crowded giving a multiplet. Also the 6.55 ppm signal attributed to the two hydrogen atoms in the methylene bridge is broad and two expected doublets are not sharp like in the other cases. The ^{13}C NMR spectrum reveals the Pd-C signal at a normal frequency of δ = 178.03 ppm. Positive ion MS (HR-ESI) analyses of **90** showed two main isotopic peaks due to the presence of bromine at *m/z* [M^+ - HBr] (732.1633 and 733.9644) attributed to the loose of one HBr molecule. Crystals suitable for X-ray crystallographic analysis were obtained by slow evaporation of acetonitrile from a concentrated warm solution.

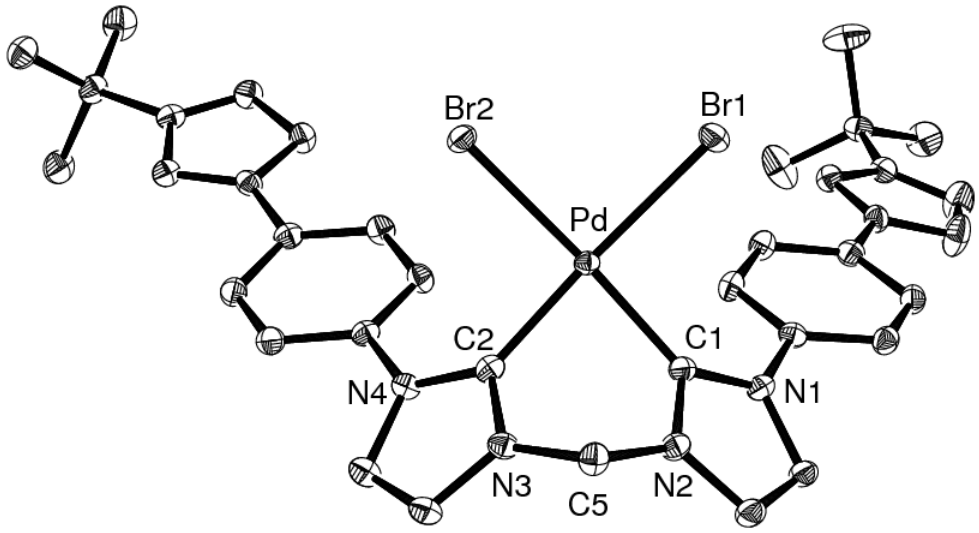


Figure 112. ORTEP representation of 1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylenediimidazolium palladium(II) dibromide (**90**) with thermal ellipsoids drawn at the 50% probability level. Hydrogen and disordered atoms were omitted for clarity. Selected bond lengths (Å) and angles (°): Pd-C(1) 1.9918(19), Pd-C(2) 1.9952(19), Pd-Br(1) 2.4814(2), Pd-Br(2) 2.4763(3), C(1)-Pd-C(2) 84.57(8), C(1)-Pd-Br(1) 92.70(5), C(2)-Pd-Br(1) 174.11(5), C(1)-N(2)-C(5) 122.41(16), N(2)-C(5)-N(3) 108.62(15), Br(1)-Pd-Br(2) 90.238(8).

The crystal structure determination of complex **90** shows that the molecule is monomeric with the dicarbene 1,2,4-oxadiazole ligand, which is chelating to the palladium(II) center in a *cis* fashion. The centered six-membered ring, C3N2Pd, has adopted a boat conformation. The bromine atoms are occupying the two remaining coordination sites of the distorted square planar coordinated palladium center.

IV.7.6. Synthesis of Nickel(II) bis-NHC complex (91)

The synthesis of 1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylenediimidazolium nickel(II) bis(hexafluorophosphate) (**91)** was performed by using the transmetallation route from the corresponding bis-silver carbene derivative in two reaction steps.

From the best of my knowledge this kind of tetra-carbenic structure is not present in the literature until this moment. Similar structures have been observed for nickel complexes of picolyl-functionalized carbene[396] and cyclophane-based nickel carbene[397] (Figure 113).

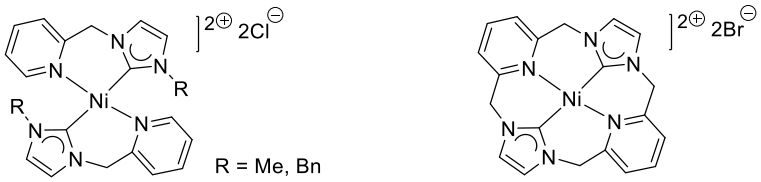
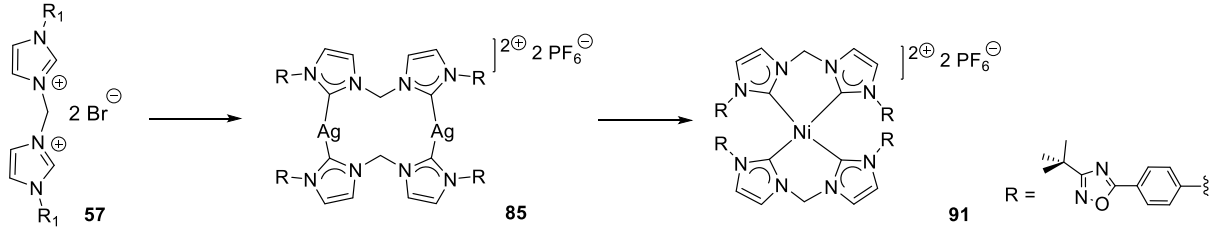


Figure 113. Examples of nickel(II) chelating complexes with N-heterocyclic carbenes.

As shown in Scheme 86, the first reaction step is the deprotonation of the 1,1′-[5-(phenyl)-3-(*tert* butyl)-1,2,4-oxadiazole]-3,3′-methylenediimidazolium bis(bromide) (**57**) by reaction with Ag_2O. In this stage is generated the Ag(I) bis-carbene complex (**85**), which can be isolated or used *in situ* after a short filtration through Celite. The second step requires the addition of $Ni(PPh_3)_2Cl_2$ in order to produce the desired nickel 1,2,4-oxadiazole complex.



Scheme 86. Preparation of nickel(II) tetra-carbene complex **91**.

Unfortunately no complete conversion was achieved even after several days of stirring or reflux. The clean product could not be isolated for accurate analyses (NMR and elemental analyses). We measured positive ion ESI-MS analysis for complexes **6** and it was observed signals belonging to the cationic complex *m/z* (M^+, 1154.44), *m/z* (M^{2+}+Ni, 606.33) and the *m/z* (M^{2+}, 548.20) as a fragments.

X-ray crystallographic analysis of nickel complex **91** confirms the presence of a mononuclear, dicationic complex with two dicarbene ligands, each of which bridges the nickel atom. (Figure 114). The molecular structure reveals a square planar geometry at the nickel atom. The tetra-carbenic complex has relatively longer Ni-C bonds (1.899(3), 1.901(3),

1.914(3), 1.909(3)), in comparison with other similar (pyridine chelating) compounds.[396,397] The two six-membered rings formed slightly distorts coordination geometry of the nickel, with C1-Ni-C2 bite angle reduced to 86.22(14)° and C3-Ni-C4 to 86.87(14)°.

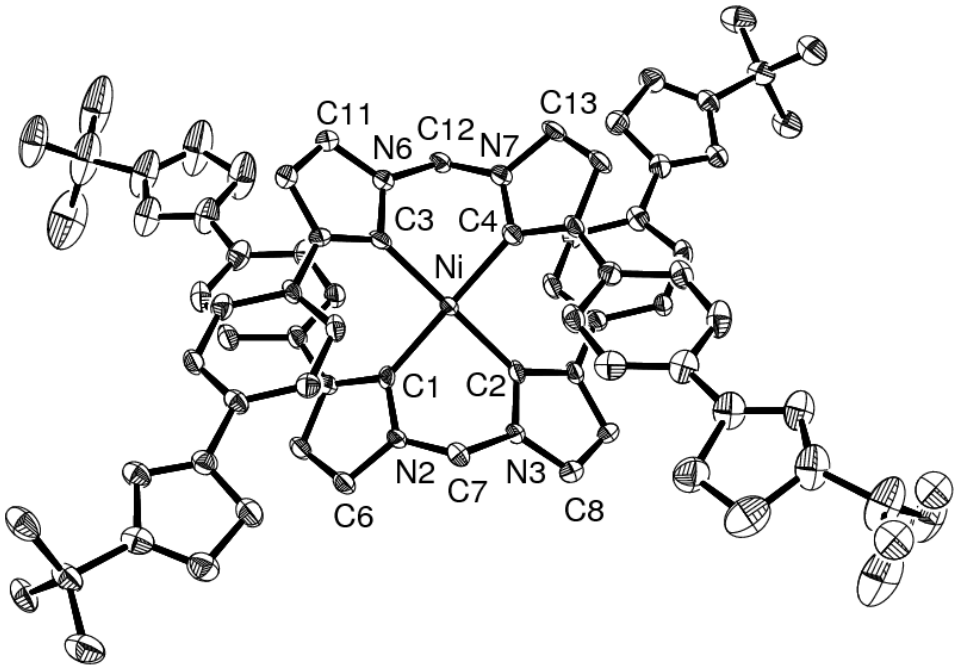


Figure 114. ORTEP representation of 1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylenediimidazolium nickel(II) bis(hexafluorophosphate) **91** with thermal ellipsoids drawn at the 50% probability level. Hydrogen, PF_6 and disordered atoms were omitted for clarity. Selected bond lengths (Å) and angles (°):Ni-C(1) 1.899(3), Ni-C(2) 1.901(3), Ni-C(3) 1.914(3), Ni-C(4) 1.909(3), C(1)-Ni-C(2) 86.22(14), C(4)-Ni-C(3) 86.87(14), C(1)-Ni-C(4) 179.67(18), C(2)-Ni-C(3) 179.60(18), C(1)-Ni-C(3) 93.41(14), C(2)-Ni-C(4) 93.50(14), C(1)-Ni-C(2)-N(3) -44.3(2), C(3)-Ni-C(1)-N(1) 40.8(3).

IV.7.7. In vitro anti-tumor activity of bridged bis-NHC complexes towards human tumor cell lines.

Complexes **83**-**86**, **88** and **90** were tested for their potential to produce a cytotoxic effect in a panel of 11 human tumor cell lines by using a monolayer cell survival and proliferation assay. In Figure 115 is presented the graphic mean IC_{50} values for the tested compounds. With a mean IC_{50} values of 5.35 μM, 5.5,44 μM and 5.99 μM, the bis-Ag(I)-NHC complex **85**, bis-Au(I)-NHC complex **86** and Rh(I)-NHC complex **84** were the most active compounds against the tested cell lines. Additionally, for **88** and **83** the anticancer activity was detected at a lower level with mean IC_{50} values of 43.43 μM to 47.2 μM. Compound **90** displayed just marginal activity.

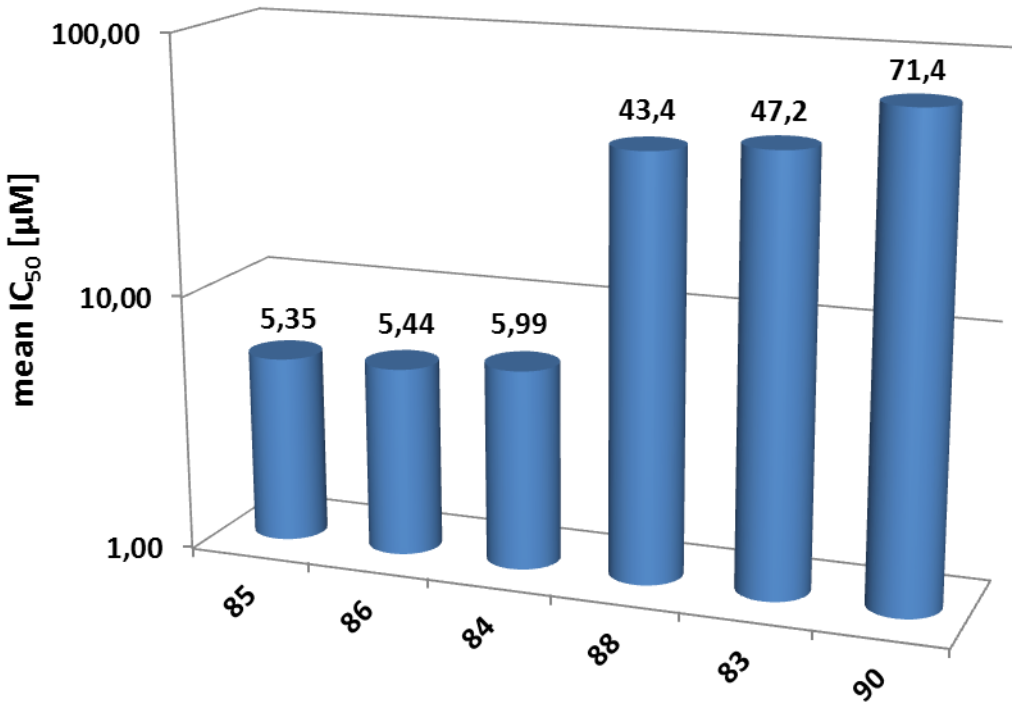


Figure 115. In vitro anti-tumor activity of compounds **83-86**, **88** and **90** in a panel of 11 human tumor cell lines (mean IC_{50} values).

Figure 116 shows the individual IC_{50} values for **83-86**, **88** and **90** as a heatmap presentation. The most active compound **85**, presented besides good activity also good selectivity against MAXF 401 (breast cancer). In the same time, the Au(I) derivative **86** was found to be selective against UXF 1138 (uterus cancer). The third most active compound, **89**, displayed individual IC_{50} values in the range from 3.11 µM (PAXF 1657) to 13.35 µM (LXFA 629), no pronounced selectivity was observed for any of the tested cell lines. Slight selectivity against GXF 251 (gastric cancer) and MAXF 401 (breast cancer) was observed for **88**. Complex **83** has also selectivity against GXF 251 (gastric cancer) and PAXF 1657 (pancreatic cancer). The palladium complex **90** showed little antitumor activity and no selectivity.

		Cell lines											
Compound	Unit	CXF HT-29	GXF 251	LXFA 629	LXFL 529	MAXF 401	MEXF 462	OVXF 899	PAXF 1657	PXF 1752	RXF 486	UXF 1138	geomean IC50 [µM]
85	µM	4,99	5,04	12,12	5,22	1,32	7,58	9,62	7,53	5,30	3,73	4,52	**5,35**
86	µM	28,94	5,47	3,94	7,90	5,27	10,50	5,27	2,27	7,85	3,86	1,25	**5,44**
84	µM	9,62	11,64	13,35	5,64	4,16	3,76	10,04	3,11	4,38	5,32	3,69	**5,99**
88	µM	37,97	17,19	63,34	40,47	19,41	55,52	100,00	61,61	39,27	52,41	45,32	**43,43**
83	µM	74,55	23,10	100,00	56,74	37,59	47,89	100,00	17,78	31,24	61,58	43,06	**47,20**
90	µM	100,00	89,30	62,40	89,80	47,50	96.0	85,40	100,00	44,00	85,20	45,30	**71,4**

1/32 1/16 1/8 1/4 1/2 1 2 4 8 16 32 -fold mean IC_{50}

sensitive cell lines — resistant cell lines

Figure 116. Heatmap presentation of individual IC_{50} values for compounds **83-86**, **88** and **90** in a panel of 11 human tumor cell lines.

IV.8. Synthesis of miscellaneous organometallic complexes containing 1,2,4-oxadiazole unit

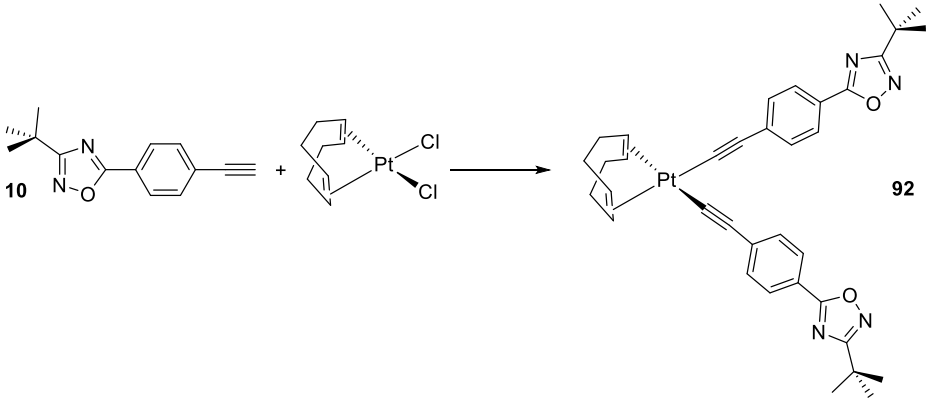
IV.8.1. Synthesis of organometallic complexes containing 1,2,4-oxadiazole alkynyl unit

IV.8.1.1. General aspects

Platinum(II) complexes containing alkynyl ligands are often employed as precursors in the synthesis of mono- and polynuclear organometallic platinum(II) compounds[398-403] or for building up homo- and heteropolymetallic compounds of platinum(II).[404] Additionally, this type of complexes can be engaged as catalysts or can be used as luminescent materials.[405-409] From the bioactivity point of view, Cullinare *et al.* reported that organometallic platinum complexes are stronger anticancer agents than the corresponding non-organometallic derivatives when tested against cancer cell lines.[410] Klein *et al*. prepared a series of [(COD)Pt(alkynyl)$_2$] and evaluate their cytotoxicity against HT-29 colon carcinoma and MCF-7 breast adenocarcinoma cell lines.[411] This type of platinum alkynyl COD complexes showed very promising activities. On the other hand, the fact that the alkynyl group is linear and that gold(I) has an good affinity for linear two coordination, makes the alkynyl gold(I) derivatives good candidates for the design of rigid-rod molecules[412] or metal-containing linear-chain polymers.[413-417] Using known and proven synthetic routes such as the ‘‘acac method’’[416] and depolymerization,[417-419] it was possible to build-up novel alkynyl gold(I) complexes derived from 3-(*tert*-butyl)-5-(4-ethynylphenyl)-1,2,4-oxadiazole.

IV.8.1.2. Synthesis of {(COD)Pt[3-(*tert*-butyl)-5-(4-ethynylphenyl)-1,2,4-oxadiazole]$_2$} (92)

The 1,2,4-oxadiazole Pt(II) complex was generated using 3-(*tert*-butyl)-5-(4-ethynylphenyl)-1,2,4-oxadiazole and [(COD)PtCl$_2$] as starting precursors. The reaction was performed in absolute ethanol in the presence of *t*-BuOK as base.

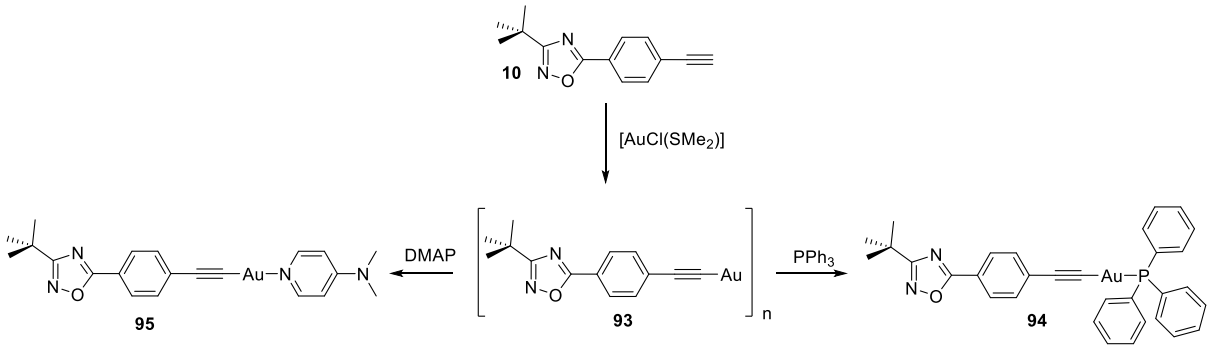


Scheme 87. COD platinum(II) complex of 3-(*tert*-butyl)-5-(4-ethynylphenyl)-1,2,4-oxadiazole.

The new complex was characterized by IR, UV/Vis, NMR spectroscopy and mass spectrometry (HR-ESI). Specific COD resonances are visible in the 1H NMR spectra as broad signals. The coupling constant $^{2}J_{PtH}$(=CH, COD) = 43 Hz suggests that the bond between Pt and the 1,2,4-oxadiazole-alkynyl ligand is rather strong. The HR-ESI measurement clearly proves the existence of the Pt(II)-complex with all isotopic signals corresponding to the mass of $M + H^+$ and $M + K^+$, respectively.

IV.8.1.3. Synthesis of ((4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)ethynyl)gold polimer (93)

The reaction between alkynyl-1,2,4-oxadiazole and [$AuCl(SMe_2)$] in the presence of trimethylamine as base, afforded the neutral oligomers as yellow solids in very good yields (Scheme 88). Because such complexes in pure state tend to explode, no further purification was performed. Also due to their insolubility in most of organic solvents, they could not be characterized by NMR techniques. The elemental analysis agrees with the calculated formula. Also in the IR spectrum is present a band at approximatively 1985 cm^{-1} corresponding to ν(C≡C) stretching mode.



Scheme 88. Synthetic route for the synthesis of 3-(*tert*-butyl)-5-(4-ethynylphenyl)-1,2,4-oxadiazole gold(I) derivatives.

IV.8.1.4. Synthesis of ((4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)ethynyl)gold(I)-PPh_3 (94) and ((4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)ethynyl)gold(I)-dimethylamino-pyridine (95) complexes

After the generation of the neutral homoleptic 1,2,4-oxadiazole-gold(I) polymer (**93**), by a depolymerization reaction using a good *σ*-donor ligand (phosphines, isocyanides or halides), novel new (alkynyl)gold(I) compounds were prepared. The solvent of choice for this type reactions is dichloromethane, in which the 1,2,4-oxadiazole-gold(I) polymer is insoluble and the new formed gold(I) alkynyl complexes are perfectly soluble. The phosphine complex **94** shows a singlet resonance at 42 ppm in the ^{31}P NMR spectrum, being in agreement with

other alkynyl(phosphine)gold(I) complexes.[420,421] The ^{1}H NMR spectra shows the resonances expected for the 1,2,4-oxadizole moiety as well as the auxiliary phosphine and dimethylaminopyridine ligands (**94** and **95**). The number of protons and relative intensities are in agreement with the proposed structures. In the ^{13}C NMR spectra the signals attributed to the Au-C appear at103 ppm for **94** and 107 ppm for **95**.

IV.8.2. Synthesis of organometallic complexes containing 1,2,4-oxadiazole thioamide unit

IV.8.2.1 General Aspects

The role of Zinc in the living processes of organisms is known to be very important especially in the catalytic and regulatory systems.[422] The synthesis of complexes from the thioamide ligands is not a very popular topic in organometallic chemistry. One inspirational example which I used is the work of Isaia *et al*.[423] in which they prepareda series of Zn complexes of Methimazole, a thioamide derivative used as antithyroid drug.[424] Of course Zn-pyrithione, a good antibacterial agent, also known to be the active ingredient in most of the anti-dandruff shampoos, is another example of medicinal marketed complexes of this kind.[425]

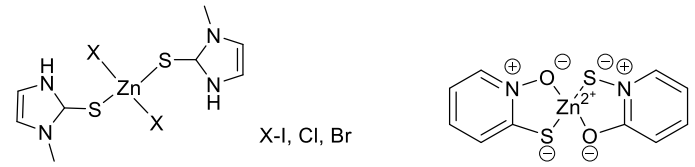
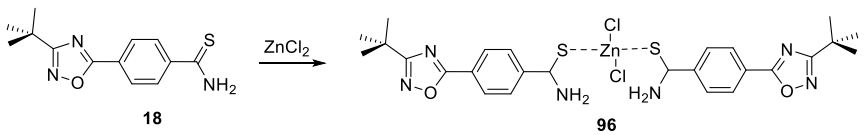


Figure 117. Examples of Zn-thione organometalic complexes.

IV.8.2.2. Synthesis of {Zn[4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)benzothioamide)]$_2$Cl$_2$} (96)

The Zn complex of 1,2,4-oxadiazole derivative was generated according to Scheme 89. The thioamide **18** was refluxed in ethanol in the presence of $ZnCl_2$ for two days. Upon cooling the solution to room temperature, the new complex precipitated in 24% yield.



Scheme 89. Synthesis of Zn-1,2,4-oxadiazole complex **96**.

Due to the low solubility of the compound no NMR or MS spectra could be collected. After several attempts, crystals suitable for X-ray determination were obtained upon cooling from a hot ethanolic solution.

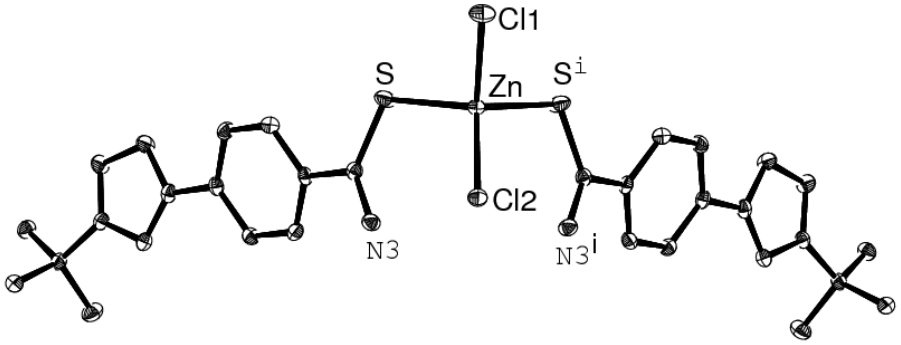


Figure 118. ORTEP representation of Zn complex (**96**) with thermal ellipsoids drawn at the 50% probability level. Hydrogen and disordered atoms were omitted for clarity. Selected bond lengths (Å) and angles (°): Zn-Cl(1) 2.2139(8), Zn-Cl(2) 2.2555(8), Zn-S 2.3702(6), Zn-S(i) 2.3702(6), Cl(1)-Zn-S 105.52(2), Cl(2)-Zn-S 113.39(18), S-Zn-S(i) 102.78(5), Cl(1)-Zn-Cl(2) 115.09(3).

The complex **96** crystallized in an orthorhombic crystal system with the space group *Pnma*. The asymmetric unit contains one molecule with a Zn^{2+} cation taking a tetrahedral geometry with two 1.2.4-oxadiazole ligands. The coordination is made via the sulfur atoms and by two Cl^- anions.

IV.8.3. *In vitro* anti-tumor activity of miscellaneous organometallic complexes containing 1,2,4-oxadiazole unit towards human tumor cell lines

In vitro anti-tumor activity of two synthesized compounds towards a panel of 12 cell lines was assessed using a monolayer cell survival and proliferation assay. By exhibiting a mean IC_{50} value of 9.3 µM **92** was the most potent compound. Compound **94** and **95** have moderate anti-tumor activity towards the 12 cell lines tested. On the opposite pole **96** exhibit just marginal activity (Figure 119).

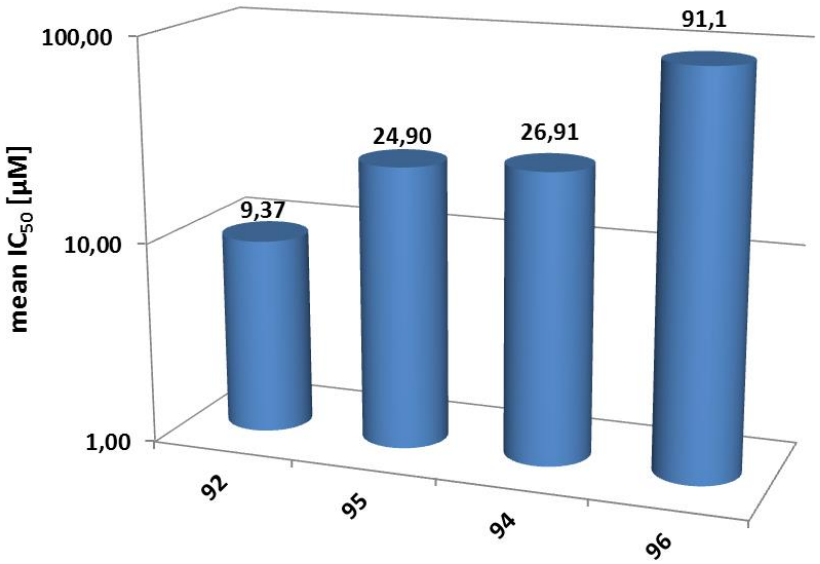


Figure 119. In vitro anti-tumor activity of compounds **92**, **94-96** in a panel of 12 human tumor cell lines (mean IC_{50} values).

Investigation of the activity of compounds in a cell line panel representing various tumour histo-types, as performed in this study, allows the analysis of potency and tumour selectivity and the identification of active compounds that qualify for further preclinical development. Tumour selectivity of the compounds is illustrated in Fig. 120, representing a heat-map presentation of the individual IC_{50} values. Overall, good antitumor potency combined with good selectivity was observed for compound **92**. Although **94** and **95** have good values for their activity, just **94** shows weak selectivity against MAXF 401 (mammary) cell lines.

compound	unit	Cell lines												Geom. mean IC_{50} [µM]
		CXF HT-29	GXF 251	LXFA 629	LXFL 529	MAXF 401	MEXF 462	OVXF 899	PAXF 1657	PRXF 22Rv1	PXF 1752	RXF 486	UXF 1138	
92	µM	40,88	7,72	16,24	11,50	1,24	2,10	35,34	8,73	14,48	7,49	10,18	8,71	**9,37**
95	µM	32,30	23,51	25,13	22,40	18,82		17,61	26,22	42.83	31,70	31,23	17.31	**24,90**
94	µM	51,65	14,37	34,11	31,27	11,79	23,80	31,73	28,72	31,65	33,85	31,93	20,85	**26,91**
96	µM	100,0	100,0	100,0	35,09	93,1	100,0	100,0	100,0	100,0	100,0	100,0	100,0	**91,10**

1/32 1/16 1/8 1/4 1/2 1 2 4 8 16 32 -fold mean IC_{50}

sensitive cell lines — resistant cell lines

Figure 120. Heatmap presentation of individual IC_{50} values for compounds **93-96** in a panel of 12 human tumor cell lines.

V. Summary and Conclusion

The starting objective of this thesis was the synthesis and antitumor evaluation of new 1,2,4-oxadiazole derivatives. This goal turned in time to the synthesis of unambiguous libraries of silver(I), gold(I), ruthenium(II) N-heterocyclic carbene complexes containing 1,2,4-oxadiazole substituents, as potential candidates for antitumor activity, which represented finally the main target of the research (Fig. 121). Compounds with various steric and electronic properties were designed, isolated and characterised by IR, ^{1}H, ^{13}C NMR, MS (HR-ESI) and X-ray diffraction.

Moreover, the biological activity of the 1,2,4-oxadiazole containing ligands and their coresponding NHC complexes was investigated. *In vitro* assessment of anti-tumor activity in a panel of 12 human tumor cell lines by a monolayer assay was evaluated.

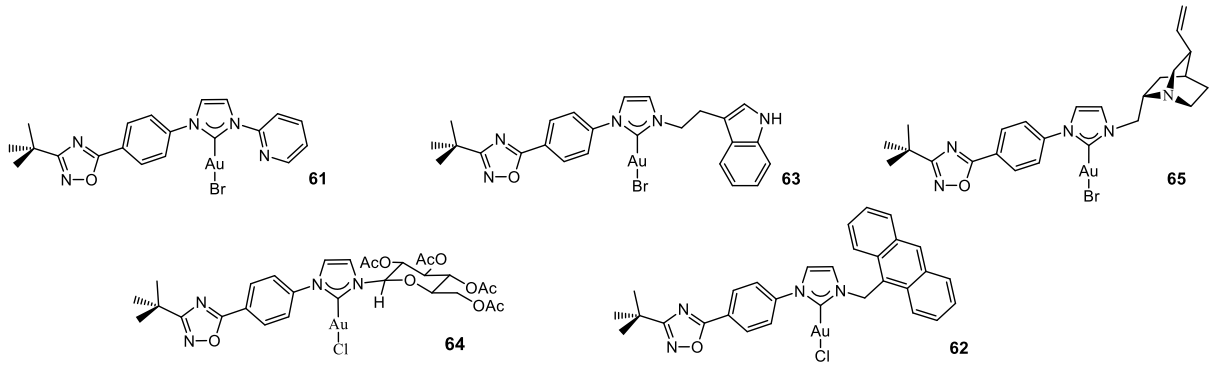


Figure 121. Representative N-heterocyclic carbene complexes containing 1,2,4-oxadiazole substituents.

First, using a one-pot methodology, it was synthesized a library of ten 3-(*tert*-butyl)-1,2,4-oxadiazoles starting from cheap and available *p*-substituted benzoic acids (I, F, OH, CN, NH_2, NO_2, aldehyde, ketone, ether, alkyne) (Fig. 122) in order to have in the 4-position of the phenyl ring a moiety that could be further modified (e.g. -NH_2 moiety for building heterocycles, -I group for cross-coupling reactions).

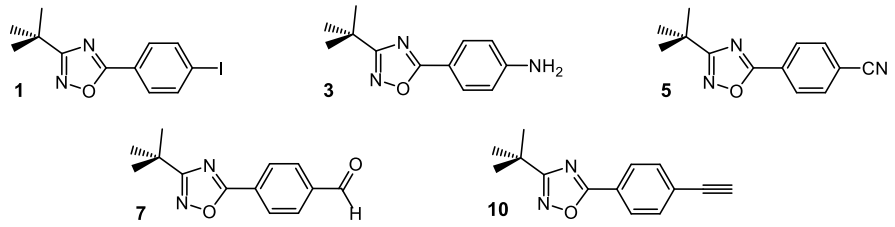
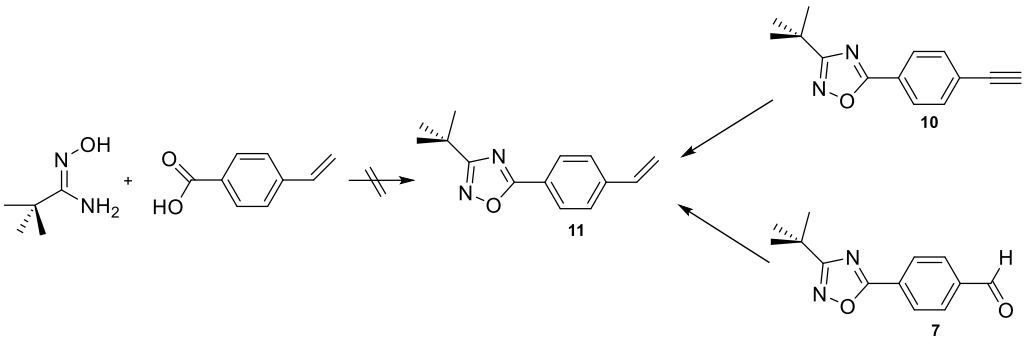


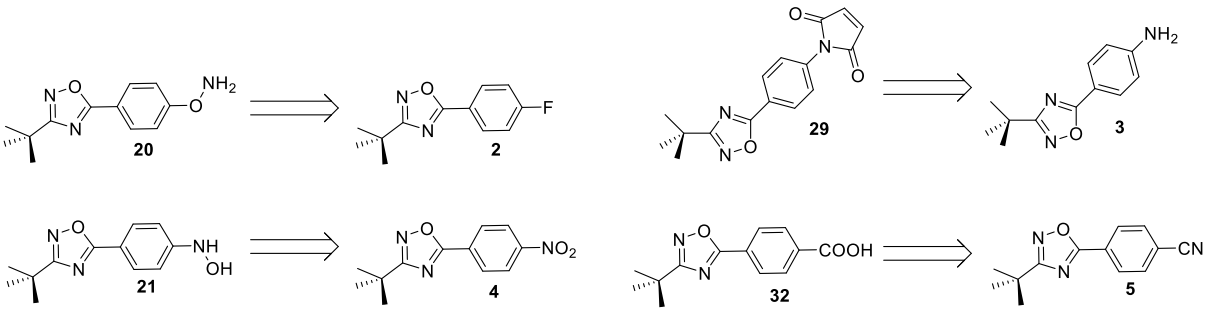
Figure 122. Representative N-heterocyclic carbene complexes containing 1,2,4-oxadiazole substituents.

Although it appears like a straightforward synthetic protocol, some *p*-substituents on the benzoic acid gave undesired reactions (e.g. in the case of a vinyl group, Scheme 90) or did not work at all.



Scheme 90. Synthetic strategies for the generation of **11**.

In the next stage these units were converted in other, more interesting moieties (e.g. carboxylic acid, succinimide, maleimide, hydrazine, hydroxyl-amines) as showed in Scheme 91.



Scheme 91. Retrosynthetic paths for the synthesis of **20**, **21**, **29** and **32**.

The synthetic plan also included the implementation of further heterocycles in order to increase the biological activity. In this direction, a series of 1,2,4-oxadiazoles incorporating a thiazole, oxazole, imidazole, pyrazole and pyrazol-pyrimidine core were designed, synthesized and investigated for antitumor activity (Fig. 123). Furthermore the results of the antitumor activity screening process guided the design of the new 1,2,4-oxadiazole compounds.

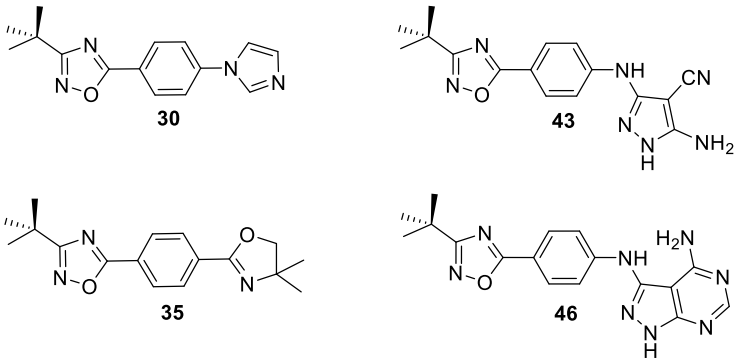


Figure 123. Heterocycles incorporated in 1,2,4-oxadiazoles compounds.

Most of the tested compounds exhibited only moderate or marginal activity against the tested cell lines and only in three cases good antitumor activity (the pyrazol-pyrimidine **47** with mean IC_{50} = 5.66 μM, the maleimide **29** with mean IC_{50} = 9.39 μM and the oxazoline **35** with mean IC_{50} = 17.39 μM) was found (Fig 124).

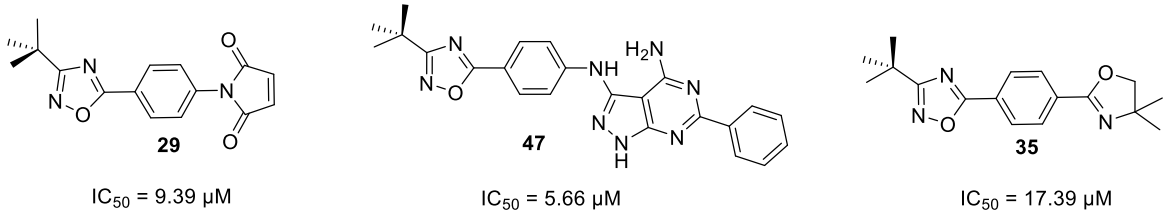
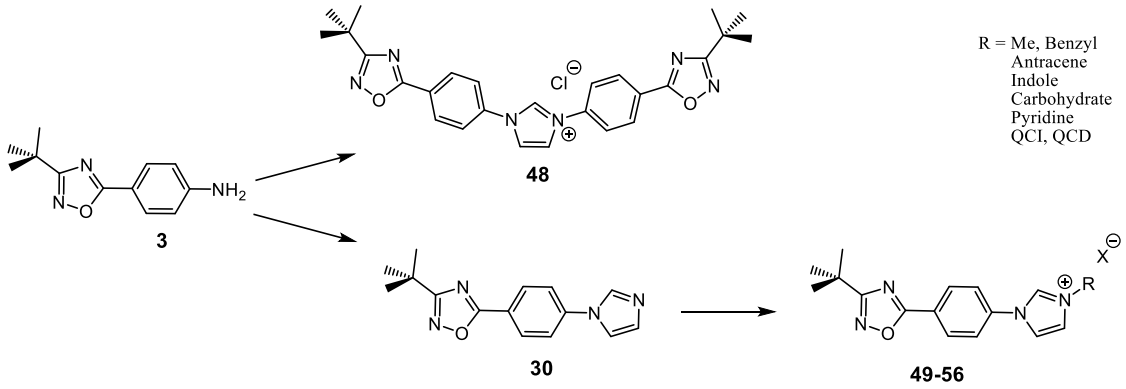


Figure 124. Best antitumoral candidates from a number of 47 synthesised 1,2,4-oxadiazole derivatives.

Based on these results we turned our focus to organometallic chemistry, more specifically to N-heterocyclic carbenes (NHC) chemistry. Starting from 1,2,4-oxadiazole derivatives substituted with imidazole or NH_2 functionalities, it was possible to generate a library of ten new imidazolium salts that were used as precursors for NHC complexes (Scheme 92).



Scheme 92. Synthetic route for the imidazolium salts **48**-**56.**

The 1,2,4-oxadiazole-derived imidazolium salts were designed mostly unsymmetrically in order to introduced a second substituent of choice. The majority of these substituents are biologically active organic moieties. Some of them are exotic (anthracene, 2-pyridine, 2,3,4,5-tetra-*O*-acetyl-*D*-glucopyranose) and others are quite unique (indole, quincorine (QCI) and quincoridine (QCD)) since no other examples are reported in the literature (Figs. 125, 126).

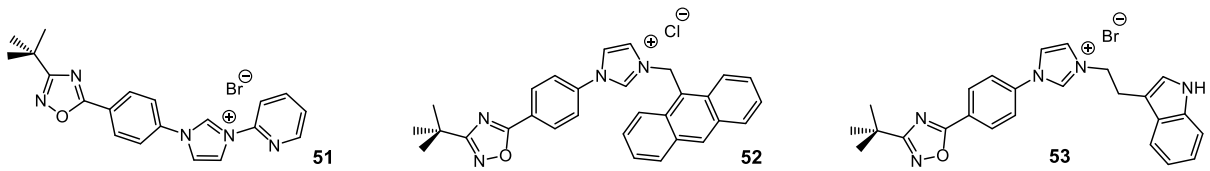


Figure 125. 1,2,4-Oxadiazole-derived imidazolium salts **51**-**53** having as second substituent pyridine, anthracene and indole moiety**.**

The inclusion of QCI, QCD and carbohydrate moieties in 1,2,4-oxadiazole based compounds conferred chiral properties and increased the selectivity of the biological activity. In addition, the attachment of glucopyranose moiety to the 1,2,4-oxadiazole conferred not only chirality, but also improved pharmacological properties (for instance better solubility in water).

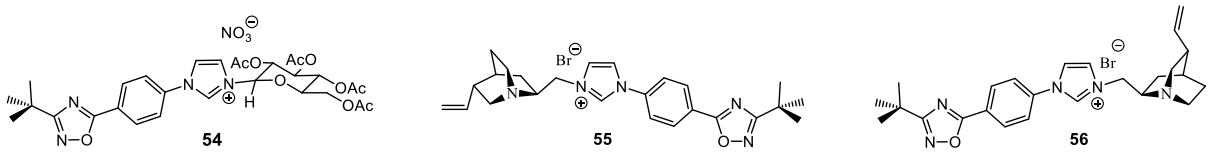


Figure 126. 1,2,4-Oxadiazole-derived imidazolium salts **54**-**56** having as second substituent glucopyranose, QCI and QCD.

The N-heterocyclic carbenes (NHC) chemistry also included the synthesis of novel metal (Ag(I), Au(I) and Rh(I)) containing NHC complexes bearing 1,2,4-oxadiazole substituents (Fig.127).

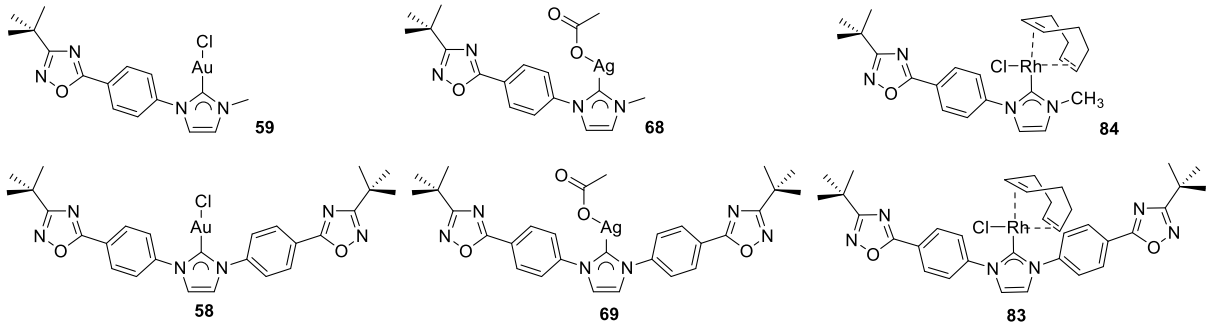


Figure 127. Examples of Ag(I), Au(I) and Rh(I)) containing NHC complexes bearing 1,2,4-oxadiazole substituents**.**

In Figure 128 are depicted six of the novel gold(I) N-heterocyclic carbenes (NHC) linked to 1,2,4-oxadiazole derivatives compounds revealed impressive potency (mean IC_{50} < 0.1 μM) and tumor selectivity, with individual IC_{50} values in the low nanomolar range, namely **61** (mean IC_{50} = 0.012 μM), **63** (0.019 μM), **64** (0.020 μM), **65** (0.049 μM), **60** (0.053 μM) and **66** (0.058 μM).

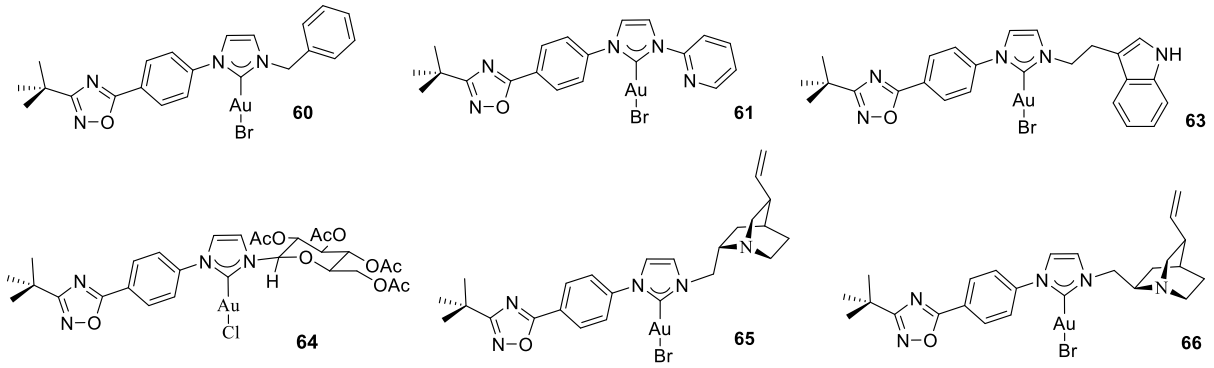
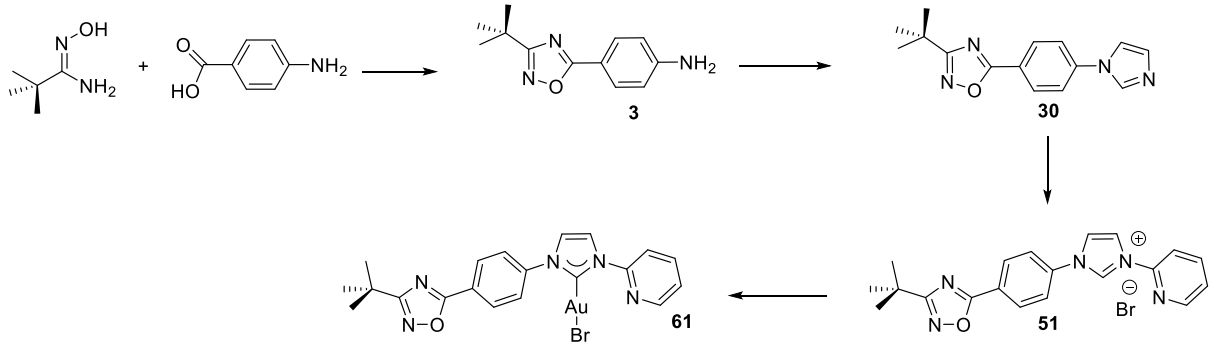


Figure 128. Gold(I)-NHC linked to 1,2,4-oxadiazole derivatives with impressive potency.

In summary, the work presented in this PhD thesis dealt with the synthesis and antitumor evaluation of 1,2,4-oxadiazole ligands and their related NHC complexes. Majority of the prepared 1,2,4-oxadiazole derivatives were tested for their antitumor activity in a monolayer cell survival and proliferation assay using human tumor cell lines of different origin/histotype. A tipically synthesis of the bioactive compounds starting from the substituted benzoic acid precursor is presented in Scheme 93.



Scheme 93. Stepwise synthesis of Au(I)-NHC derivative **61** starting from the benzoic acid precursor.

VI. Outlook

The work presented in this PhD thesis gives important contribution to three traditional disciplines, namely organic synthesis, inorganic synthesis and medicinal chemistry. Combining the features of this complementary areas leads to overcome barriers and increase the liberty of designing *de novo* bioactive compounds. But at the end of the work still remain some opened challenges.

The synthesis of platinum NHC complexes of 1,2,4-oxadiazole-2-ylidene class and their biological evaluation remains an open chapter. This type of complexes could not be generated (Fig 129).

The synthesis of iridium NHC complexes containing 1,2,4-oxadiazole ligands also remained unconquered territory although several synthetic methods were employed (Fig 129).

Figure 129. Unexplored Pt and Ir *NHC* complexes containing 1,2,4-oxadiazole ligands.

Building-up "sweet carbenes" - NHC complexes substituted with carbohydrate moieties in both (1 and 3) positions of the imidazolium core remains a big challenge especially because nobody managed to obtain this kind of sugar carbenes. The small number of publications related to sugar-NHC complexes, present just NHC-sugar examples mono-substituted.

Building-up "bitter carbenes" - NHC complexes substituted with quinuclidine moieties (QCI or QCD) in both positions of the imidazolium core represent even a bigger challenge. Although the sensitivity of these special moieties will make the synthesis ambitious, the final compounds could lead to new strong biologically active drugs (Fig 130).

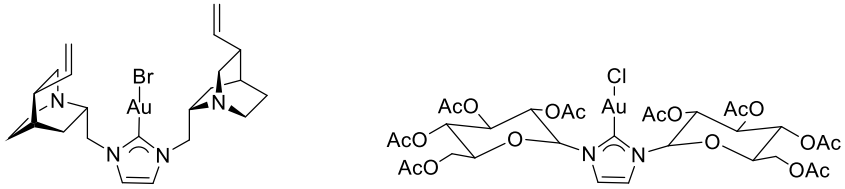


Figure 130. New challenging compounds.

Finally, the tendency to make laboratory innovations better and integrate them into real-live applications, represent the power-source of chemical advance. In the same time, the curiosity-driven research and serendipity will continue to be a powerful weapon in the fight between chemical boundaries and mysteries of nature.

VII. Experimental section

General Procedures. All reagents were purchased from commercial sources (Sigma-Aldrich or Acros) and used without further purification. Solvents were of analytical grade. ^{1}H and ^{13}C NMR spectra were recorded at room temperature on a Bruker Avance 300 operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Chemical shifts (δ) are reported relative to the tetramethylsilane peak (δ = 0.00 ppm). IR spectra were recorded with a Bruker Vertex 70 ATR. Mass spectra were recorded on a Finnigan MAT 8400-MSS and Finnigan MAT 4515. High resolution mass spectra were recorded on a Finnigan MAT 95 XP. For elemental C, H, N analyses VarioMICRO V3.1.1. were used. All complexes reported in the manuscript **13-24** have a purity of >95%. The purity of compounds **2-12** was ascertained to be >95% by HPLC on Shimadzu CBM-10A instrument with a Pinnacle DB C18 (5µm, 250 x 4.6 mm) column using $(NEt_3H)H_2PO_4$ buffer (0.08M in H_2O) / 95% MeCN with $(NEt_3H)H_2PO_4$ buffer (0.08M in H_2O) gradients as the eluents. Reactions were monitored by TLC, performed on silica gel plates 40 x 80 mm Polygram Sil G\UV$_{254}$ (Macherey-Nagel). Visualization on TLC was achieved by UV light. Column chromatography was performed with Merck silica gel 60 (70–200 mesh).

***In vitro* antitumor activity towards human tumor cell lines**

Antitumor activity of these compounds was tested in a monolayer cell survival and proliferation assay using human tumor cell lines. Studies using panels of human tumor cell lines of different origin/histotype allow for analysis of potency and tumor selectivity of test compounds.

Ten out of the twelve cell lines as tested were established at Oncotest from patient-derived human tumor xenografts passaged subcutaneously in nude mice. The origin of the donor xenografts has been described. The cell line 22RV1 was supplied by ATCC ((Rockville, MD), HT-29 was kindly provided by the National Cancer Institute (Bethesda, MA, USA). Cells were cultured in RPMI 1640 medium, supplemented with 10% fetal calf serum and 0.1 mg/mL gentamicin under standard conditions (37 °C, 5% CO_2). Authenticity of all cell lines was proven by STR analysis at the DSMZ (Braunschweig, Germany).

A modified propidium iodide assay was used to assess activity of the compounds toward human tumor cell lines. Briefly, cells were harvested from exponential phase cultures by trypsinization, counted and plated in 96-well flat-bottom microtiter plates at a cell density dependent on the cell line (4.000–20.000 cells/well). After a 24 h recovery period to allow the

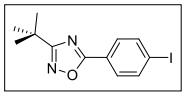
cells to adhere and resume exponential growth, compounds were added at 10 concentrations in half-log increments and left for a further 4 d. The inhibition of proliferation was determined by measuring the DNA content using an aqueous propidium iodide solution (7 µg/mL). Fluorescence was measured using the Enspire Multimode-Plate Reader (excitation λ = 530 nm, emission λ = 620 nm), providing a direct relationship to the total viable cell number. In each experiment, all data points were determined in duplicates. Anti-tumor activity was reported as the absolute IC_{50} value, which reflects the concentration of the test compound that achieves test/control values of 50%. Calculation was performed using a four-parameter non-linear curve fit (Oncotest Data Warehouse Software). The overall potency of a compound was determined by the geometric mean IC_{50} values of all individual IC_{50} values. In the heatmap representation of IC_{50} values, the distribution of the IC_{50} values obtained for a test compound in the individual tumor models is given in relation to the geometric mean IC_{50} value, obtained over all cell lines tested.

The individual IC values were highlighted in colors ranging from dark green (≤1/32-fold geometric mean IC_{50}, corresponds to very potent compound activity or high tumor sensitivity) to dark red (≥32-fold geometric mean IC_{50}, corresponds to lack of compound activity or tumor resistance). The heatmap presentation, therefore, represents an anti-proliferative “fingerprint” profile of a test compound.

Abbreviations **Abbreviations in NMR-Spectroscopy**

THF	tetrahydrofuran	δ	chemical shift
DCM	dichloromethane	ppm	parts per million
DMF	dimethylformamide	s	singulet
CDI	carboxyldiimidazol	d	doublet
NHC	N-heterocyclic carbene	t	triplet
IC_{50}	half maximal inhibitory concentration	q	quartet
DMS	dimethyl sulfide	m	multiplet
THT	tetrahydrothiophene	br	broad
Me	methyl	*o*	*ortho*
t-Bu	*tert*-butyl	*m*	*meta*
Ph	phenyl	*p*	*para*
min	minute		
h	hour		
eq.	equivalent		
r.t.	room temperature		
calc.	calculated		

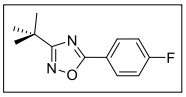
Synthesis of 3-*tert*-butyl-5-(4-iodophenyl)-1,2,4-oxadiazole (1)



A solution of 4-iodobenzoic acid (15.0 g, 0.06 mol) in DMF (200 mL) was treated with a solution of CDI (10.78 g, 0.066 mol) in DMF (150 mL). After 30 minutes of stirring at room temperature, a solution of *tert*-butylamidoxime (7.66 g, 0.066 mol) in DMF (60 mL) was added and the reaction mixture was stirred for one hour at room temperature. A second portion of CDI (10.78 g, 0.066 mol.) solved in DMF (150 mL) was added and the mixture was heated to reflux for 3 hours. The mixture was cooled to room temperature and poured into a water-ice mixture. The solid thus formed was filtered off, washed with water, dried and flash chromatographed with ethyl acetate/hexane. Yield*:* 85% (16.73 g, 0.051 mol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.96 – 7.79 (m, 4H, Ar-*H*), 1.42 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.19 (Cq, N*C*O), 175.25 (Cq, N*C*N), 138.31 (*C*H), 129.39 (*C*H), 124.06 (Cq), 99.62 (Cq), 32.53 (Cq, *C*$(CH_3)_3$), 28.48((*C*$H_3)_3$C) ppm. MS: *m*/*z* = 328.0 (M^+, 70), 313.0 (20), 230.0 (100).

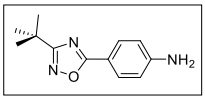
Synthesis of 3-*tert*-butyl-5-(4-fluorophenyl)-1,2,4-oxadiazole (2)



A solution of 4-fluorobenzoic acid (15.0 g, 0.107 mol) in DMF (200 mL) was treated with a solution of CDI (19.09 g, 0.117 mol) in DMF (150 mL). After 30 minutes of stirring at room temperature, a solution of *tert*-butylamidoxime (13.59 g, 0.117 mol) in DMF (60 mL) was added and the reaction mixture was stirred for one hour at room temperature. A second portion of CDI (19.09 g, 0.117 mol) solved in DMF (150 mL) was added and the mixture was heated to reflux for 3 hours. The mixture was cooled to room temperature and poured into a water-ice mixture. The solid thus formed was filtered off, washed with water, dried and flash chromatographed with ethyl acetate/hexane. Yield*:* 45% (10.61 g, 0.048 mol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.22 – 8.08 (m, 2H, Ar-*H*), 7.28 – 7.13 (m, 2H, Ar-*H*), 1.43 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.37 (Cq, N*C*O), 174.19 (Cq, N*C*N), 167.77 (Cq, *C*F), 130.35 (*C*H), 121.04 (Cq), 116.47 (*C*H), 32.48 (Cq, *C*$(CH_3)_3$), 28.43 ((*C*$H_3)_3$C) ppm. MS: *m*/*z* = 220.1 (M^+, 30), 205.1 (24), 123.1 (100).

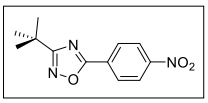
Synthesis of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-*yl*)aniline (3)



A solution of 4-aminobenzoic acid (1.0 g, 7.29 mmol) in DMF (20 mL) was treated with a solution of CDI (1.28 g, 7.91 mmol) in DMF (30 mL). After 30 minutes of stirring at room temperature, a solution of *tert*-butylamidoxime (0.91 g, 7.91 mmol) in DMF (20 mL) was added, and the reaction mixture was stirred for one hour at room temperature. A second portion of CDI (1.28 g, 7.91 mmol.) solved in DMF (30 mL) was added and the mixture was heated to reflux for 3.5 hours. The mixture was cooled to room temperature and poured into a water-ice mixture. The solid thus formed was filtered off, washed with water, dried and flash chromatographed with ethyl acetate/hexane. Yield*:* 59% (0.92 g, 4.2 mmol).

IR (ATR): 1/λ 3437 (w), 3326 (w), 3218 (w), 2966 (w), 2360 (w), 2162 (w), 1699 (w), 1652 (w), 1603 (s), 1581 (m), 1522 (w), 1510 (m), 1491 (m), 1463 (m), 1442 (m), 1415 (w) 1394 (w), 1351 (s), 1329 (w), 1291 (w), 1197 (m), 1186 (s), 1172 (s), 1096 (w), 1028 (w), 962 (w), 845 (m), 835 (m), 777 (s), 700 (w), 645 (s) cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.87 – 7.78 (m, 2H, Ar-*H*), 6.68 – 6.56 (m, 2H, Ar-*H*), 4.08 (bs, 2H, N*H*$_2$), 1.32 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 177.34 (Cq, N*C*O), 174.76 (Cq, N*C*N), 149.76 (Cq, *C*NH_2), 129.18 (*C*H), 113.76 (*C*H), 113.55 (Cq), 31.72 (Cq, *C*$(CH_3)_3$), 27.86 ((*C*H_3)$_3$C) ppm. MS: *m*/*z* = 217 (M^+, 60), 202 (5), 120 (100); HRMS calcd. for $C_{12}H_{15}N_3O^+$: 217.12096; found 217.12159. M.p. 120-122°C.

Synthesis of 3-*tert*-butyl-5-(4-nitrophenyl)-1,2,4-oxadiazole (4)

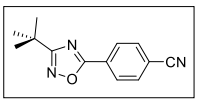


To a mixture of 0.116 g (1 mmol) of *tert*-butylamidoxime in acetonitrile (5 mL) was added 0.150 g (1 mmol) of 4-nitrobenzonitrile. To this reaction mixture was added 0.07 g (0.3 mmol) of 2-mesitylenesulfonic acid and 0.067 g (0.3 mmol) of $ZnBr_2$ and the mixture was heated to 80ºC for 2 hours. After the reaction was finished, the mixture was cooled to room temperature. The solvent was removed and ethyl acetate (20 mL) was added. The mixture was washed with sodium hydrogen carbonate solution, water and brine. The organic phase was dried over anhydrous sodium sulfate, filtered and the solvent was removed. The resulting residue was dried in vacuum and flash chromatographed (silica, ethyl acetate/hexane 2:1). Yield*:* 94% (0.232 g, 0.94 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.43 – 8.28 (m, 4H, Ar-*H*), 1.45 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.44 (Cq, N*C*O), 174.26 (Cq, N*C*N), 148.96 (Cq, *C*NO_2),

144.68 (*C*H), 129.86 (*C*H), 124.25 (Cq), 32.62 (Cq, *C*$(CH_3)_3$), 28.46 ((*C*$H_3)_3$C-) ppm. MS: *m*/*z* = 247 (M^+, 25), 232 (45), 150 (100).

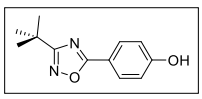
Synthesis of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)benzonitrile (5)



A solution of 4-cyanobenzoic acid (50.0 g, 0.340 mol) in DMF (500 mL) was treated with a solution of CDI (1,1'-carbonyldiimidazole) (60.55 g, 0.374 mol) in DMF (400 mL). After 30 min stirring at room temperature, a solution of *tert*-butylamidoxime (43.38 g, 0.374 mol) in DMF (200 mL) was added and the reaction mixture was stirred for 1 h at room temperature. A second portion of CDI (60.55 g, 0.374 mol) dissolved in DMF (400 mL) was added and the mixture was heated to reflux for 5 h. The mixture was cooled to room temperature and poured into a water-ice mixture. The solid thus formed was filtered off, washed with water, dried and flash chromatographed with ethyl acetate/hexane. Yield*:* 76% (58.65 g, 0.258 mol). Crystals suitable for X-ray diffraction analysis were formed by slow evaporation of a chloroform solution at room temperature.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.32 – 8.19 (m, 2H, Ar-*H*), 7.97 – 7.78 (m, 2H, Ar-*H*), 1.43 (s, 9H, *t*-Bu). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.48 (Cq, N*C*O), 174.96 (Cq, N*C*N), 132.76 (*C*H), 128.58(*C*H), 127.89 (Cq), 119.89 (Cq, *C*N), 111.82 (Cq, *C*CN) 32.62 (Cq, *C*$(CH_3)_3$), 28.43 ((*C*$H_3)_3$C). MS: *m*/*z* = 227.1 (M^+, 65), 212.1 (40), 130.1 (100).

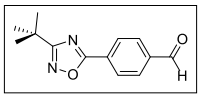
Synthesis of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-*yl*)phenol (6)



A solution of 4-hydroxybenzoic acid (5.0 g, 0.036 mol) in DMF (50 mL) was treated with a solution of CDI (6.45 g, 0.040 mol) in DMF (40 mL). After 30 minutes of stirring at room temperature, a solution of *tert*-butylamidoxime (4.64 g, 0.040 mol) in DMF (20 mL) was added and the reaction mixture was stirred for one hour at room temperature. A second portion of CDI (6.45 g, 0.040 mol.) solved in DMF (40 mL) was added and the mixture was heated to reflux for 6 hours. The mixture was cooled to room temperature and poured into a water-ice mixture. The solid thus formed was filtered off, washed with water, dried and flash chromatographed with ethyl acetate/hexane. Yield*:* 64% (5.02 g, 0.023 mol).

^{1}H NMR (200 MHz, DMSO-d6): δ = 10.45 (broad signal, -O*H*), 7.98 – 7.86 (m, 2H, Ar-*H*), 7.01 – 6.88 (m, 2H, Ar-*H*), 1.33 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, DMSO-d6): δ = 177.42 (Cq, N*C*O), 174.73 (Cq, N*C*N), 161.64 (Cq, *C*OH), 129.78 (*C*H), 116.14 (*C*H), 114.39 (Cq), 31.94 (Cq, *C*$(CH_3)_3$), 28.10 ((*C*$H_3)_3$C) ppm. MS: *m*/*z* = 218.2 (M^+, 60), 203.2 (20), 121.1 (100).

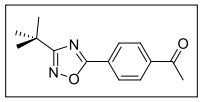
Synthesis of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-*yl*)benzaldehyde (7)



A solution of 4-formylbenzoic acid (15.0 g, 0.1 mol) in DMF (150 mL) was treated with a solution of CDI (17.8 g, 0.11 mol) in DMF (100 mL). After 30 minutes of stirring at room temperature, a solution of *tert*-butylamidoxime (12.66 g, 0.11 mol) in DMF (60 mL) was added and the reaction mixture was stirred for one hour at room temperature. A second portion of CDI (17.8 g, 0.11 mol.) solved in DMF (100 mL) was added and the mixture was heated to reflux for 4 hours. The mixture was cooled to room temperature and poured into a water-ice mixture. The solid thus formed was filtered off, washed with water, dried and flash chromatographed with ethyl acetate/hexane. Yield*:* 72% (16.57 g, 0.072 mol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 10.11 (s, 1H, CO*H*), 8.38 – 8.26 (m, 2H, Ar-*H*), 8.09 – 7.98 (m, 2H, Ar-*H*), 1.44 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 191.28 (*C*H, *C*OH), 165.68 (Cq, N*C*O), 156.55 (Cq, N*C*N), 138.70 (Cq), 130.08(*C*H), 129.13 (Cq), 128.70 (*C*H), 32.61 (Cq, *C*$(CH_3)_3$), 28.46((*C*H_3)$_3$C) ppm. MS: *m*/*z* = 230.1 (M^+, 38), 215.1 (30), 133.1 (100).

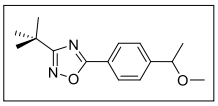
Synthesis of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-*yl*)phenyl)ethanone (8)



A solution of 4-acetylbenzoic acid (10.0 g, 0.061 mol) in DMF (150 mL) was treated with a solution of CDI (10.86 g, 0.067 mol) in DMF (100 mL). After 30 minutes of stirring at room temperature, a solution of *tert*-butylamidoxime (7.78 g, 0.067 mol) in DMF (60 mL) was added and the reaction mixture was stirred for one hour at room temperature. A second portion of CDI (10.86 g, 0.067 mol.) solved in DMF (100 mL) was added and the mixture was heated to reflux for 2 hours. The mixture was cooled to room temperature and poured into a water-ice mixture. The solid thus formed was filtered off, washed with water, dried and flash chromatographed with ethyl acetate/hexane. Yield*:* 96% (14.28 g, 0.058 mol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.28 – 8.19 (m, 2H, Ar-*H*), 8.16 – 8.05 (m, 2H, Ar-*H*), 2.67 (s, 3H, C*H*$_3$), 1.35 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 197.87 (Cq, *C*O), 174.66 (Cq, N*C*O), 158.75 (Cq, N*C*N), 141.26 (Cq), 129.45 (*C*H), 129.03 (Cq), 128.96 (*C*H), 31.65 (Cq, *C*$(CH_3)_3$), 29.12 ((*C*H_3)$_3$C), 27.89 (-*C*H_3) ppm. MS: *m*/*z* = 244.1 (M^+, 38), 229.1 (100), 147.1 (40).

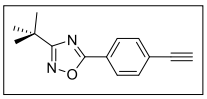
Synthesis of 3-*tert*-butyl-5-(4-(1-methoxyethyl)phenyl)-1,2,4-oxadiazole (9)



A solution of 4-(1-methoxyethyl)benzoic acid (10.0 g, 0.055 mol) in DMF (150 mL) was treated with a solution of CDI (9.9 g, 0.061 mol) in DMF (100 mL). After 30 minutes of stirring at room temperature, a solution of *tert*-butylamidoxime (7.02 g, 0.061 mol) in DMF (60 mL) was added and the reaction mixture was stirred for one hour at room temperature. A second portion of CDI (9.9 g, 0.061 mol.) solved in DMF (100 mL) was added and the mixture was heated to reflux for 4 hours. The mixture was cooled to room temperature and poured into a water-ice mixture. The solid thus formed was filtered off, washed with water, dried and flash chromatographed with ethyl acetate/hexane. Yield: 92% (13.16 g, 0.05 mol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.95 – 7.98 (m, 2H, Ar-*H*), 7.44 – 7.32 (m, 2H, Ar-*H*), 4.29 (m, 1H, C*H*), 3.17 (s, 3H, C*H*$_3$), 1.38 (m, 3H, C*H*$_3$), 1.35 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 177.69 (Cq, N*C*O), 174.35 (Cq, N*C*N), 147.86 (Cq), 127.66 (*C*H), 126.04 (*C*H), 123.13 (Cq), 78.58 (Cq), 55.99 (O*C*H$_3$), 31.85 (Cq, *C*(CH$_3$)$_3$), 27.87((*C*H$_3$)$_3$C) ppm. MS: *m*/*z* = 260.2 (M^+, 10), 245.2 (100), 189.2 (42).

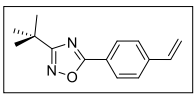
Synthesis of 3-*tert*-butyl-5-(4-ethynylphenyl)-1,2,4-oxadiazole (10)



A solution of *p*-ethynylbenzoic acid (1.5 g, 10.273 mmol) in DMF (10 mL) was treated with a solution of CDI (1.83 g, 11.301 mmol) in DMF (10 mL). After 30 minutes of stirring at room temperature, a solution of *t*-butylamidoxime (1.3 g, 11.301 mmol) in DMF (5 mL) was added and the reaction mixture was stirred for one hour at room temperature. A second portion of CDI (1.83 g, 11.301 mmol.) solved in DMF (10 mL) was added and the mixture was heated to reflux for 3.5 hours. The mixture was cooled to room temperature and poured into a water-ice mixture. The solid thus formed was filtered off, washed with water, dried and flash chromatographed with ethyl acetate/hexane. Yield*:* 86% (2.0 g, 8.834 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.13 – 8.05 (m, 2H, Ar-*H*), 7.66 – 7.58 (m, 2H, Ar-*H*), 3.26 (s, 1H, C*H*$_{Alkin}$), 1.43 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.50 (Cq, N*C*O), 174.41 (Cq, N*C*N), 132.64 (*C*H), 127.94 (*C*H), 126.28 (Cq), 124.59 (Cq), 82.69 (Cq), 80.25 (*C*H), 32.54 (Cq, *C*(CH$_3$)$_3$), 28.48 ((*C*H$_3$)$_3$C) ppm. MS: *m*/*z* = 226.1 (M^+, 50), 221.1 (20), 129.1 (100).

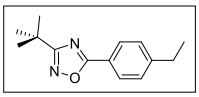
Synthesis of of 3-*tert*-butyl-5-(4-vinylphenyl)-1,2,4-oxadiazole (11)



Method 1: 5 g of 3-*tert*-butyl-5-(4-ethynylphenyl)-1,2,4-oxadiazole (22.10 mmol) was dissolved in 150 mL of THF and cooled to -10ºC. To this solution 0.5 g of Lindlar catalyst was added and hydrogen was bubbled through the mixture for 30 minutes. The progress of the reaction was monitored by ^{1}H NMR. The mixture was filtered over silica gel, the silica gel was washed with diethyl ether and the organic phases were evaporated yielding 4.8 g (21.43 mmol, 97%) of a mixture 3-*tert*-butyl-5-(4-ethynylphenyl)-1,2,4-oxadiazole 90% and 3-*tert*-butyl-5-(4-ethylphenyl)-1,2,4- oxadiazole (7%) as a colorless solid. *Method 2*: 5 g of 4-(3-*tert*-butyl-1,2,4- oxadiazol-5-yl)-benzaldehyde (21.74 mmol), 3.3 g of 1,8-Diazabicyclo[5.4.0]undec-7-ene (21.74 mmol) and 7.76 g of methyltriphenylphosphine bromide (21.74 mmol) were dissolved in 200 mL of THF and heated to 50ºC for 3 hours. The reaction mixture was filtered over silica gel, the silica gel was washed with diethyl ether and the organic phases were evaporated. The product was flash chromatographed with ethyl acetate/hexane yielding 3.87 g (16.95 mmol, 78%)

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.13 – 8.02 (m, 2H, Ar-*H*), 7.57 – 7.46 (m, 2H, Ar-*H*), 6.75 (dd, *J* = 17.6, 10.9 Hz, 1H, CH=C*H*$_2$), 5.87 (dd, *J* = 17.5, 0.6 Hz, 1H, CH=C*H*$_2$), 5.44 – 5.33 (m, 1H, C*H*=CH_2), 1.43 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.31 (Cq, N*C*O), 174.85 (Cq, N*C*N), 141.41 (Cq), 135.85 (CH, *C*H=CH_2), 128.28 (*C*H), 126.64 (*C*H), 123.69 (Cq), 116.46 (CH_2, CH=*C*H_2), 30.28 (Cq, *C*$(CH_3)_3$), 28.47 ((*C*H_3)$_3$C) ppm. MS: *m*/*z* = 228.1 (M^+, 50), 213.1 (20), 131.1 (100).

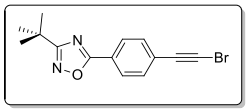
Synthesis of 3-*tert*-butyl-5-(4-ethylphenyl)-1,2,4- oxadiazole (12)



5 g of 3-*tert*-butyl-5-(4-ethynylphenyl)-1,2,4-oxadiazole (22.10 mmol) was dissolved in 150 mL of THF. To this solution 0.5 g of Lindlar catalyst was added and hydrogen was bubbled through the solution for 1 hour. The reaction progress was monitored by ^{1}H-NMR. The mixture was filtered over silica gel, the silica gel was washed with diethyl ether and the organic phases were evaporated yielding 4.98 g (21.66 mmol, 98%)

^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.91 – 7.83 (m, 2H, Ar-H), 7.20 – 7.09 (m, 2H, Ar-*H*), 2.55 (q, *J* = 7.6 Hz, 2H, C*H*$_2$), 1.31 (s, 9H, *t*-Bu), 1.18 – 1.03 (m, 3H, C*H*$_3$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 177.60 (Cq, N*C*O), 174.59 (Cq, N*C*N), 148.54 (Cq), 127.79 (*C*H), 127.48 (*C*H), 121.48 (Cq), 31.81 (Cq, *C*$(CH_3)_3$), 28.31 (*C*H_2CH_3), 27.84 ((*C*H_3)$_3$C); 14.51 (CH_2*C*H_3) ppm. MS: *m*/*z* = 230.2 (M^+, 40), 215.2 (M^+, 20), 133.2 (M^+, 100).

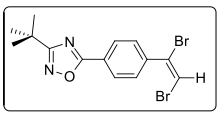
Synthesis of 5-(4-(bromoethynyl)phenyl)-3-*tert*-butyl-1,2,4-oxadiazole (13)



A solution of 200 mg of 3-*tert*-butyl-5-(4-ethynylphenyl)-1,2,4-oxadiazole (0.885 mmol) in acetone (10 mL) was treated with 60 mg of $AgNO_3$ (0.353 mmol). The mixture was cooled to 0ºC and 157 mg of NBS (0.885 mmol) solved in acetone (10 mL) was dropped slowly. The reaction mixture was stirred for 24 hours at room temperature. The solvent was evaporated and the residue was flash chromatographed with ethyl acetate/hexane. Yield*:* 63% (170 mg, 0.557 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.16 – 8.03 (m, 2H, Ar-*H*), 7.68 – 7.51 (m, 2H, Ar-*H*), 1.43 (s, 9H, *t*-Bu). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 179.12 (Cq, N*C*O), 176.14 (Cq, N*C*N), 133.15 (*C*H), 128.59 (*C*H), 127.47 (Cq), 125.03 (Cq), 80.59 (Cq, *C*≡CBr), 54.31 (Cq, C≡*C*Br), 33.16 (Cq, *C*$(CH_3)_3$), 29.10 ((*C*$H_3)_3$C). MS: *m*/*z* = 304.0 (M^+, 50), 305.0 (M^+, 15), 306.0 (M^+, 55), 307.0 (M^+, 10), 289.0 (15), 290.0 (5), 291.0 (15), 292.0 (5), 209 (80), 207.0 (100).

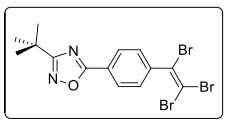
Synthesis of (E)-3-*tert*-butyl-5-(4-(1,2-dibromovinyl)phenyl)-1,2,4-oxadiazole (14)



A solution of 200 mg of 3-*tert*-butyl-5-(4-ethynylphenyl)-1,2,4-oxadiazole (0.885 mmol) in acetone (10 mL) was treated with 60 mg of $AgNO_3$ (0.353 mmol). The mixture was cooled to 0ºC and 393.82 mg of NBS (2.21 mmol) solved in acetone (10 mL) was dropped slowly. The reaction mixture was stirred for 24 hours at room temperature. The solvent was evaporated and the residue was flash chromatographed with ethyl acetate/hexane. Yield*:* 43% (147 mg, 0.380 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.18 – 8.07 (m, 2H, Ar-*H*), 7.72 – 7.62 (m, 2H, Ar-*H*), 7.23 (s, H, C*H*Br), 1.44 (s, 9H, *t*-Bu). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.72 (Cq, N*C*O), 176.34 (Cq, N*C*N), 140.47 (Cq), 128.30 (*C*H), 128.29 (*C*H), 125.31 (Cq, *C*Br), 123.13 (Cq), 111.06 (*C*HBr), 33.26 (Cq, *C*$(CH_3)_3$), 28.48 ((*C*$H_3)_3$C). MS: *m*/*z* = 384.0 (M^+, 45), 385.0 (M^+, 80), 388.0 (M^+, 45), 369.0 (15), 371.0 (25), 373.0 (15), 286.9 (65), 288.9 (100), 290.9 (55).

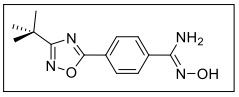
Synthesis of 3-*tert*-butyl-5-(4-(1,2,2-tribromovinyl)phenyl)-1,2,4-oxadiazole (15)



A solution of 200 mg of 3-*tert*-butyl-5-(4-ethynylphenyl)-1,2,4-oxadiazole (0.885 mmol) in acetone (10 mL) was treated with 60 mg of $AgNO_3$ (0.353 mmol). The mixture was cooled to 0ºC and 393.82 mg of NBS (2.21 mmol) solved in acetone (10 mL) was dropped slowly. The reaction mixture was stirred for 24 hours at room temperature. The solvent was evaporated and the residue was flash chromatographed with ethyl acetate/hexane. Yield*:* 26% (107 mg, 0.230 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.14 – 8.04 (m, 2H, Ar-*H*), 7.53 – 7.42 (m, 2H, Ar-*H*), 1.36 (s, 9H, *t*-Bu). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.93 (Cq, N*C*O), 176.14 (Cq, N*C*N), 143.47 (Cq), 129.46 (*C*H), 128.26 (*C*H), 125.61 (Cq, *C*Br), 122.73 (Cq), 93.36 (Cq, *C*Br_2), 33.56 (Cq, *C*$(CH_3)_3$), 28.46 ((*C*$H_3)_3$C). MS: *m*/*z* = 461.9-467.9 (M^+, 80), 364.8-370.8 (100).

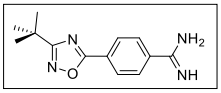
Synthesis of (*Z*)-4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)-*N*'-hydroxybenzimidamide (16)



15.28 g of hydroxylamine hydrochloride (220 mmol) and 30.4 g of anhydrous potassium carbonate (220 mmol) were dissolved in 200 mL of dry isopropanol and stirred for 0.5 h at room temperature. The 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)benzonitrile (10 g, 44 mmol) was added, and the reaction mixture was heated to reflux 12 h. After filtration of inorganic salts, the solvent was evaporated under reduced pressure. The product was purified by column chromatography (petroleum ether/acetone, 2:1, v/v). Yield*:* 64% (7.33 g, 28 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.23 – 8.12 (m, 2H, Ar-*H*), 7.86 – 7.75 (m, 2H, Ar-*H*), 5.04 – 4.78 (bs, 2H, NH_2), 1.71 – 1.53 (bs, O*H*), 1.44 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.68 (Cq, N*C*O), 170.67 (Cq, N*C*N), 152.57 (Cq, N*C*N), 136.11 (Cq), 128.38 (*C*H), 126.29 (*C*H), 125.45 (Cq), 32.56 (Cq, *C*$(CH_3)_3$), 28.50 ((*C*$H_3)_3$C) ppm. MS: *m*/*z* = 260.1 (M^+, 100), 243.1 (50), 130.1 (80).

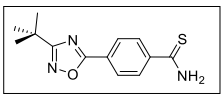
Synthesis of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-*yl*)benzimidamide (17)



To 3g of (*Z*)-4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)-*N*'-hydroxy-benzimidamide (11.52 mmol) dissolved in glacial HOAc (50 mL) was added slowly 3.63 g of ammonium formate (57.62 mmol) and 1.5 g Pd/C (10%) and the mixture was heated at reflux until consumption of the starting material. The mixture was cooled and filtered through Celite. The filtrate was evaporated, and the residue was basified with aq NaOH (1 M) and extracted with EtOAc (3×250 mL). The combined organic layers were washed with H_2O (5×200 mL), brine and dried over Na_2SO_4. Yield*:* 69% (1.93 g, 7.94 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.23 – 8.11 (m, 2H, Ar-*H*), 7.83 – 7.71 (m, 2H, Ar-*H*), 4.95 – 5.45 (bs, 3H, N*H*, NH_2), 1.44 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.46 (Cq, N*C*O), 174.66 (Cq, NH_2*C*NH), 164.63 (Cq, N*C*N), 140.02 (Cq), 128.36 (*C*H), 126.71 (*C*H), 126.17 (Cq), 32.47 (Cq, *C*$(CH_3)_3$), 28.40 ((*C*$H_3)_3$C) ppm. MS (ESI): 245.13 [100, M+H^+], 246.13 [18].

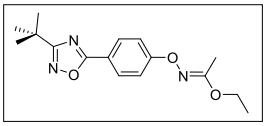
Synthesis of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)benzothioamide (18)



To a solution of 4.48 g of NaSH (80 mmol, 68 % in H_2O) and 8.13 g of $MgCl_2.6H_2O$ (40 mmol) in DMF (90 mL) was added 9 g of 4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)benzonitrile (40 mmol). The reaction was stirred at room temperature for 4 hours. Water (300 mL) was added and the precipitate thus formed was filtrated and washed with 500 mL 1N HCl, H_2O and then was dried in vacuum to afford 9.6 g of yellow powder. Yield*:* 92% (9.6 g, 36.8 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.24 – 8.13 (m, 2H, Ar-*H*), 8.06 – 7.95 (m, 2H, Ar-*H*), 7.82 – 7.67 (bs, S*H*), 7.33 – 7.18 (bs, N*H*), 1.44 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 187.63 (Cq, S*C*NH_2), 176.56 (Cq, N*C*O), 164.66 (Cq, N*C*N), 141.22 (Cq), 128.81 (*C*H), 128.08 (*C*H), 126.47 (Cq), 31.27 (Cq, *C*$(CH_3)_3$), 29.12 ((*C*H_3)$_3$C) ppm. MS: *m*/*z* = 261.1 (M^+, 40), 227.1 (20), 164.1 (40), 130.1 (20).

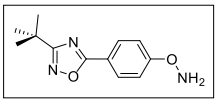
Synthesis of (*E*)-ethyl N-4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenoxyacetimidate (19)



To a solution of 5.12 g of (*Z*)-ethyl *N*-hydroxyacetimidate (49.6 mmol) in DMF (40 mL) was added at 0ºC 6.12 g of *t*-BuOK (54.6 mmol). After stirring the reaction mixture for 60 min at room temperature, 12.0 g of 3-*tert*-butyl-5-(4-fluorophenyl)-1,2,4-oxadiazole (54.6 mmol) was added and the reaction mixture was heated at 80ºC for 2.5 h. The reaction was cooled to 0ºC and H_20 (250 mL) was added. The aqueous layer was extracted with ethyl acetate (300 mL) and the combined organic layer was washed with brine (150 mL), dried over N_2SO_4, and concentrated. The residue was chromatographed on a column of silica gel with ethyl acetate-hexane. Yield*:* 68% (11.26 g, 37.12 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.12 – 8.01 (m, 2H, Ar-*H*), 7.32 – 7.20 (m, 2H, Ar-*H*), 4.28 – 4.15 (q, 2H, C*H*$_2$), 2.14 (s, 3H, C*H*$_3$), 1.42 (s, 9H, *t*-Bu), 1.37 (t, 3H, C*H*$_3$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.19 (Cq, N*C*O), 175.09 (Cq, N*C*(CH_3)O), 166.45 (Cq, N*C*N), 162.92 (Cq, *C*NO), 129.71 (*C*H), 117.59 (Cq), 114.15 (*C*H), 63.18 (CH_2, O*C*H_2CH_3), 32.45 (Cq, *C*$(CH_3)_3$), 28.52 ((*C*H_3)$_3$C), 14.41 (C*C*H_3), 14.32 (CH_3, O-CH_2-*C*H_3) ppm. MS: *m*/*z* = 303.1 (M^+, 90), 215.1 (50), 121.1 (100).

Synthesis of *O*-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)hydroxylamine (20)

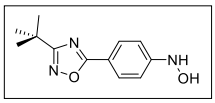


To a solution of 9.09 g of (*E*)-ethyl N-4-(3-*tert*-butyl-1,2,4-oxadiazol-5-

yl)phenoxyacetimidate (30.0 mmol) in dioxane (7.5 mL) cooled at 0ºC was added dropwise 70% $HClO_4$, (22.17 mL). After stirring the reaction mixture for 3 h at room temperature, the mixture was poured into ice-water (750 mL). The aqueous layer was made basic by addition of NaOH and it was extracted with ethyl acetate (250 mL x 3). The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was chromatographed on a column of silica gel with ethyl acetate-hexane (2: 1). Yield*:* 51% (3.56 g, 15.3 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.11 – 7.97 (m, 2H, Ar-*H*), 7.33 – 7.19 (m, 2H, Ar-*H*), 6.11 – 5.82 (bs, N*H*$_2$), 1.42 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.18 (Cq, N*C*O), 175.02 (Cq, N*C*N), 164.61 (Cq, *C*NO), 129.71 (*C*H), 117.57 (Cq), 113.54 (*C*H), 32.43 (Cq, *C*$(CH_3)_3$), 28.49 ((*C*$H_3)_3$C) ppm. MS: *m*/*z* = 233.1 (M^+, 30), 218.1 (40), 121.1 (100).

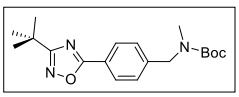
Synthesis of *N*-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)hydroxylamine (21)



To a solution of 6.18 g of 3-*tert*-butyl-5-(4-nitrophenyl)-1,2,4-oxadiazole (25.0 mmol) in ethanol (75 mL) and water (40 mL) was added 1.57 g of $BiCl_3$ (10.0 mmol). To this mixture, 5.39 g of KBH_4 (100.0 mmol) was added gradually with stirring under an atmosphere of nitrogen. After the addition, the mixture was stirred at room temperature for 15 minutes. Then, under bubbling of nitrogen, the mixture was acidified to pH 7 with 1N HCl and immediately extracted with Et_2O. The extract was washed with brine and dried over anhydrous Na_2S0_4. After removing the solvent under reduced pressure the residue was chromatographed on a column of silica gel with ethyl acetate-hexane.Yield*:* 76% (4.43 g, 19.0 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.09 – 7.94 (m, 2H, Ar-*H*), 7.14 – 6.96 (m, 3H, Ar-*H*, O*H*), 6.49 (bs, N*H*), 1.43 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 177.46 (Cq, N*C*O), 174.62 (Cq, N*C*N), 153.10 (Cq, *C*NHOH), 128.72 (*C*H), 116.77 (Cq), 112.95 (*C*H), 31.81 (Cq, *C*$(CH_3)_3$), 27.82 ((*C*$H_3)_3$C) ppm. MS: *m*/*z* = 233.1 (M^+, 25), 218.1 (50), 136.1 (20), 120.1 (100).

Synthesis of *tert*-butyl 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)benzyl(methyl)carbamate (22)

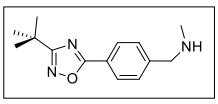


A solution of 5 g of 4-((methyl(pivaloyloxy)amino)methyl)benzoic acid (18.84 mmol) in DMF (28 mL) was treated with a solution of CDI (3.36 g, 20.77 mmol) in DMF (22.2 mL). After 30 minutes of stirring at room temperature, a solution of *tert*-butylamidoxime (2.41 g, 20.77 mmol) in DMF (10 mL) was added and the

reaction mixture was stirred for one hour at room temperature. A second portion of CDI (3.36 g, 20.77 mmol.) solved in DMF (22.2 mL) was added and the mixture was heated to reflux for 2 hours. The mixture was cooled to room temperature and poured into a water-ice mixture. The solid thus formed was filtered off, washed with water, dried and flash chromatographed with ethyl acetate/hexane. Yield*:* 86% (5.59 g, 16.2 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.18 – 8.04 (m, 2H, Ar-*H*), 7.44 – 7.28 (m, 2H, Ar-*H*), 4.49 (s, 2H, C*H*$_2$), 2.87 (s, 3H, C*H*$_3$), 1.47 (s, 9H, *t*-Bu), 1.43 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.31 (Cq, N*C*O), 174.89 (Cq, N*C*N), 143.03 (Cq, N*C*OO), 128.31 (*C*H), 127.72 (Cq), 123.54 (Cq), 79.96 (Cq, O*C*(CH$_3$)$_3$), 53.93 (Ph*C*H$_2$N), 34.22 (Cq, *C*(CH$_3$)$_3$), 32.46 (N*C*H$_3$), 28.45 ((*C*H$_3$)$_3$C), 28.37 ((*C*H$_3$)$_3$C) ppm. MS: EI *m*/*z* = 345.2 (M^+, 10), 330.2 (20), 289.2 (70), 244.2 (100), 203.2 (90).

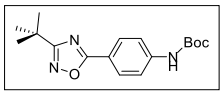
Synthesis of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-*N*-methylmethanamine (23)



A solution of 5 g of tert-butyl 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)benzyl(methyl)carbamate (14.47 mmol) in Et_2O (250 mL) was cooled to 0ºC and 22 mL of a 2N solution of HCl (43.4 mmol) in Et_2O was added dropwise. The solid thus formed was filtered off, washed with Et_2O, dried and dissolved in H_2O. The pH was adjusted to basic value and the solution was extracted with ethyl acetate. After the separation of the two phases the organic one was dried over Na_2SO_4 and the solvent was removed under reduced pressure. Yield*:* 96% (3.4 g, 13.89 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.14 – 8.05 (m, 2H, Ar-*H*), 7.53 – 7.42 (m, 2H, Ar-*H*), 3.84 (s, 2H, C*H*$_2$), 2.47 (s, 3H, C*H*$_3$), 1.43 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.33 (Cq, N*C*O), 175.07 (Cq, N*C*N), 145.54 (Cq), 128.61 (*C*H), 128.18 (*C*H), 123.35 (Cq), 55.61 (Ph*C*H$_2$N), 35.96 (Cq, *C*(CH$_3$)$_3$), 32.49 (HN*C*H$_3$), 28.49 ((*C*H$_3$)$_3$C) ppm. MS: EI *m*/*z* = 245.2 (M^+, 40), 230.2 (10), 203.2 (100).

Synthesis of *tert*-butyl 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylcarbamate (24)

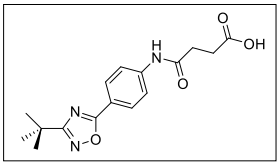


To a solution of 1.5 g of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)aniline (6.9 mmol) in THF (50 mL) was added 276.17 g of NaOH (7.0 mmol) dissolved in 20 mL of water. The mixture was cooled at 0°C when 1.5 g of Boc_2O (6.9 mmol) was added. The reaction mixture was warmed to room temperature and was stirred for 24h. Afterwards the THF was removed under reduced pressure. The aqueous residue was extracted two times with Et_2O. After the separation of the two phases the organic one was dried over

Na_2SO_4 and the solvent was removed under reduced pressure. The residue was loaded on a silica column. (Et_2O:Hexane // 2:1). Yield: 88% (1.92 g, 6.07 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.03 – 7.89 (m, 2H, Ar-*H*), 7.49 – 7.38 (m, 3H, Ar-*H*), 6.88 (bs, N*H*), 1.43 (s, 9H, *t*-Bu), 1.43 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 177.56 (Cq, N*C*O), 174.16 (Cq, N*C*N), 151.58 (Cq, NH*C*OO), 141.72 (Cq), 128.54 (*C*H), 118.20 (Cq), 117.36 (*C*H), 80.58 (Cq, O*C*$(CH_3)_3$), 31.79 (Cq, *C*$(CH_3)_3$), 27.84 ((*C*$H_3)_3$C), 27.59 ((*C*$H_3)_3$C-) ppm. MS (ESI): 318.2 (25, M + H^+), 340.2 (100, M + Na^+), 657.4 (75, 2 x M + Na^+).

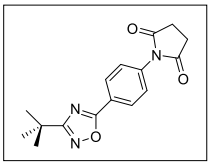
Synthesis of 4-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylamino)-4-oxobutanoic acid (25)



0.3 g (1.38 mmol) of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)aniline was mixed under inert conditions with 0.138 g (1.38 mmol) of succinic anhydride in 20 mL of DCM. This mixture was vigorously stirred at room temperature for 5 hours. The remaining residue was freed from solvent, suspended in 50 mL of water and the pH value adjusted to 2-3 with a 1 N solution of HCl. The resulting suspension was filtered and the product was dried in vacuum. The residue was purified by flash chromatography. Yield: 91% (0.398 g, 1.25 mmol).

IR (ATR): 1/λ 3385 (w), 2964 (w), 1696 (s), 1608 (m), 1594 (w), 1506 (s) 1492 (m), 1464 (w), 1410 (m)1394 (w), 1351 (m), 1337 (m), 1313 (m), 1281 (w), 1249 (w), 1200 (m), 1177 (m), 1159 (s), 994 (w), 859 (m), 830 (w), 802 (w), 773 (m), 681 (m), 612 (m) cm^{-1}. ^{1}H NMR (200 MHz, DMSO-d_6): δ = 12.24 (bs, 1H, -COO*H*), 10.42 (s, 1H, -N*H*), 8.07 – 8.02 (m, 2H, Ar-*H*), 7.92-7.96 (m, 2H, Ar-*H*), 2.67-2.59 (m, 4H, 2 x CH_2), 1.38 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, DMSO-d_6): δ = 177.51 (Cq, NH*C*O), 174.35 (Cq, N*C*O), 173.63 (Cq, *C*OOH), 170.75 (Cq, N*C*N), 143.31 (Cq), 128.70 (*C*H), 118.89 (*C*H), 117.61 (Cq), 31.96 (Cq, *C*$(CH_3)_3$), 31.12 (*C*H_2), 28.53 (*C*H_2), 28.46 ((*C*$H_3)_3$C) ppm. MS: *m*/*z* = 317 (M^+, 15), 299 (55), 217 (50), 202 (100), 120 (55). HR-MS calcd. for $C_{16}H_{19}N_3O_4^+$: 317.13701; found 317.13717. M.p. 201-203°C.

Synthesis of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)pyrrolidine-2,5-dione (26)

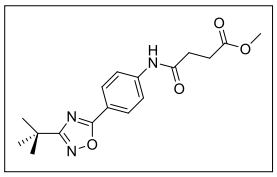


0.3 g (0.94 mmol) of 4-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylamino)-4-oxobutanoic acid was mixed with 0.077 g of sodium acetate (0.94 mmol) in 15 mL of acetic anhydride, and the mixture was then heated for 4 h at 80-

85ºC. After cooling, the product was washed with 100 mL of H_2O at pH ≈ 2-3. The solid was dried in vacuum and flash chromatographed. Yield: 87% (0.244 g, 0.81 mmol,).

IR (ATR): 1/λ 2970 (w), 2875 (w), 1776 (w), 1709 (s), 1613 (w), 1520 (w), 1500 (w), 1465 (w), 1419 (w), 1382 (m), 1351 (w), 1275 (w), 1197 (m), 1171 (s), 923 (w), 904 (w), 841 (m), 810 (w), 771 (w), 737 (w), 697 (m), 659 (w) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = (200 MHz, $CDCl_3$): 8.18 – 8.09 (m, 2H, Ar-*H*), 7.48-7.39 (m, 2H, Ar-*H*), 2.80 (s, 4H, 2 x C*H*$_2$), 1.34 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.34 (Cq, N*C*O), 175.53 (Cq, *C*=O), 174.04 (Cq, N*C*N), 135.35 (Cq), 128.65 (*C*H), 126.63 (*C*H), 124.20 (Cq), 32.39 (Cq, *C*$(CH_3)_3$), 29.51(*C*H_2), 28.31 ((*C*H_3)$_3$C) ppm. MS: *m*/*z* = 299 (M^+, 55), 284 (15), 202 (100). HR-MS calcd. for $C_{16}H_{17}N_3O_3^+$: 299.12644; found 299.12695. M.p. 136-138°C.

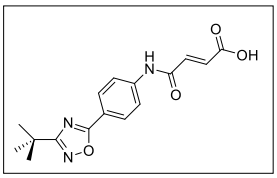
Synthesis of methyl 4-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylamino)-4-oxobutanoate (27)



A suspension of 0.3 g (0.94 mmol) of 4-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylamino)-4-oxobutanoic acid in 10 mL of diethyl ether was cooled in an ice bath and an solution of diazomethane in diethyl ether 2.5 N was added dropwise until the colorless solid disappeared and the gas evolution stopped. The solvent was removed under high vacuum. No further purification was necessary. Yield: 96% (0.298 g, 0.9 mmol).

IR (ATR): 1/λ 3391 (w), 2966 (w), 1738 (m), 1702 (m), 1607 (m), 1595 (w), 1507 (s), 1492 (m), 1524 (w), 1392 (w), 1351 (m), 1327 (s), 1274 (w), 1251 (w), 1197 (m), 1172 (s), 1156 (s), 1097 (w), 991 (w), 988 (w), 863 (m), 836 (w), 797 (w), 773 (m), 699 (w), 690 (w), 617 (s) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 8.34 (s, 1H, -N*H*), 8.11 – 8.02 (m, 2H, Ar-*H*), 7.70-7.66 (m, 2H, Ar-*H*), 3.72 (s, 3H, C*H*$_3$), 2.82-2.67 (m, 4H, 2 x C*H*$_2$) 1.42 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 177.62 (Cq, NH*C*O), 173.97 (Cq, N*C*O), 173.05 (Cq, *C*OOMe), 169.52 (Cq, N*C*N), 141.06 (Cq), 128.43 (*C*H), 119.31 (Cq), 118.77 (*C*H), 51.43 (CH_3, O*C*H_3), 31.79 (*C*H_2), 28.38 (*C*H_2), 28.34 (Cq, *C*$(CH_3)_3$), 27.80 ((*C*H_3)$_3$C) ppm. MS: *m*/*z* = 331 (M^+, 10), 299 (50), 284 (15), 202 (100). HR-MS calcd. for $C_{17}H_{21}N_3O_4^+$: 331.15266; found 331.15275.

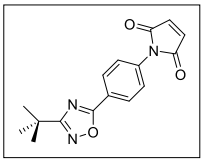
Synthesis of (*Z*)-4-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylamino)-4-oxobut-2-enoic acid (28)



0.3 g (1.38 mmol) of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)aniline was mixed under inert conditions with 0.135 g (1.38 mmol) of maleic anhydride in 20 mL of DCM. This mixture was vigorously stirred at room temperature for 3 hours. The remaining residue was freed from solvent and suspended in 50 mL of water, and the pH value was adjusted to 2-3 using a 1 N solution of HCl. The resulting suspension was filtered and the solid obtained was dried in vacuum and flash chromatographed. Yield: 84% (0.365 g, 1.15 mmol).

IR (ATR): 1/λ 3288 (w), 3104 (w), 2974 (w), 1707 (m), 1633 (w), 1616 (w), 1586 (m), 1566 (w), 1532 (s), 1520 (s), 1496 (s), 1467 (m), 1409 (w), 1396 (w), 1348 (w), 1325 (m), 1281 (w), 1267 (w), 1188 (m), 1101 (w), 976 (m), 899 (w), 849 (s), 776 (m), 737 (w), 738 (w), 632 (w), 615 (m) cm^{-1}. ^{1}H NMR (200 MHz, DMSO-d_6): δ = 12.94 (bs, 1H, -COO*H*), 10.73 (s, 1H, -N*H*), 8.14 – 8.03 (m, 2H, Ar-*H*), 7.96-7.85 (m, 2H, Ar-*H*), 6.61-6.32 (m, 2H, 2 x C*H*), 1.38 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, DMSO-d_6): δ = 177.56 (Cq, NH*C*O), 174.28 (Cq, N*C*O), 166.80 (Cq, *C*OOH), 163.67 (Cq, N*C*N), 142.77 (Cq), 131.50 (*C*H=CH), 130.19 (CH=*C*H), 128.73 (*C*H), 119.44 (*C*H), 118.30 (Cq), 31.98 (Cq, *C*$(CH_3)_3$), 28.07 ((*C*H_3)$_3$C) ppm. MS: *m*/*z* = 315 (M^+, 15), 297 (50), 217 (55), 200 (100), 120 (75). HR-MS calcd. for $C_{16}H_{17}N_3O_4^+$: 315.12136; found 315.12144. M.p. 214-216°C.

Synthesis of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-1H-pyrrole-2,5-dione (29)

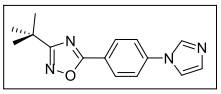


0.25 g (0.79 mmol) of (*Z*)-4-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylamino)-4-oxobut-2-enoic acid was mixed with 0.065 g of sodium acetate (0.79 mmol) in 15 mL of acetic anhydride, and the mixture was then heated for 4 h at 80-85ºC. After cooling, the product was washed with 100 mL of water at pH ≈ 2-3. The solid was dried in vacuum and flash chromatographed. Yield: 75% (0.176 g, 0.59 mmol).

IR (ATR): 1/λ 3474 (w), 3096 (w), 2975 (w), 2927 (w), 1713 (s), 1614 8w), 1567 (w), 1521 (m), 1502 (m), 1466 (w), 1395 (m), 1385 (m), 1351 (w), 1306 (w), 1238 (w), 1195 (m), 1149 (s), 1064 (w), 1022 (w), 988 (w), 949 (w), 905 (w), 842 (m), 827 (s), 771 (m), 737 (w), 700 (s), 686 (m), 652 (w), 624 (w) cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.26 – 8.14 (m, 2H, Ar-*H*), 7.62-7.54 (m, 2H, Ar-*H*), 6.88 (s, 2H, 2 x C*H*), 1.44 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.21 (Cq, N*C*O), 174.03 (Cq, N*C*N), 168.66 (Cq, *C*O), 134.87 (Cq),

134.21 (Cq), 128.58 (*C*H), 125.54 (*C*H), 123.24 (Cq), 32.31 (Cq, *C*(CH_3)$_3$), 28.27 ((*C*H_3)$_3$C) ppm. MS: *m*/*z* = 297 (M^+, 60), 282 (15), 200 (100). HR-MS calcd. for $C_{16}H_{15}N_3O_3^+$: 297.11079; found 297.11127. M.p. 123-125°C.

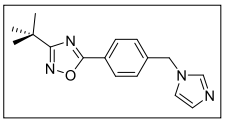
Synthesis of 5-(4-(1H-imidazol-1-*yl*)phenyl)-3-*tert*-butyl-1,2,4-oxadiazole (30)



4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)aniline (10.0 g, 0.046 mol) in MeOH (20 mL) was treated with 40% aqueous glyoxal (5.25 mL, 0.046 mol) at room temperature for 10 min to form a yellow mixture. NH_4Cl (4.92 g, 0.092 mol) was added followed by 37% aqueous formaldehyde (7.37 mL, 0.092 mol). The mixture was diluted with MeOH (100 mL) and heated to reflux for 1 h before H_3PO_4 (6.45 mL, 85%) was slowly added. The resulting mixture was then stirred at reflux for another 12 h. After removal of the solvent, the dark residue was poured onto ice (300 g) and stirred at room temperature for 1 hour. The suspension was filtered and the filtrate was made basic to pH 12 with NaOH solution. The colorless solid thus formed was filtered, washed with plenty of water and dried. Yield: 48% (5.9 g, 0.022 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.33 – 8.21 (m, 2H, Ar-*H*), 7.97 (s, 1H, CH_{Im}), 7.62 – 7.53 (m, 2H, Ar-*H*), 7.42 -7.36 (m, 1H, CH_{Im}), 7.28 -7.25 (m, 1H, CH_{Im}), 1.45 (s, 9H, *t*-Bu). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 179.13 (Cq, N*C*O), 174.54 (Cq, N*C*N), 140.85 (Cq), 135.96 (CH, N*C*HN), 131.81 (CH, N*C*HCH), 130.51 (*C*H), 124.10 (Cq), 121.82 (*C*H), 118.29 (CH, CH*C*HN), 33.14 (Cq, *C*(CH_3)$_3$), 29.06 ((*C*H_3)$_3$C). MS: *m*/*z* = 268.1 (M^+, 90), 253.1 (20), 171 (100).

Synthesis of 5-(4-((1H-imidazol-1-*yl*)methyl)phenyl)-3-*tert*-butyl-1,2,4-oxadiazole (31)

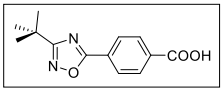


(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-*yl*)phenyl)methanamine (1.07 g, 0.0046 mol) in MeOH (2 mL) was treated with 40% aqueous glyoxal (0.525 mL, 0.0046 mol) at room temperature for 10 min to form a yellow mixture. NH_4Cl (0.492 g, 0.0092 mol) was added followed by 37% aqueous formaldehyde (0.737 mL, 0.0092 mol). The mixture was diluted with MeOH (10 mL) and heated to reflux for 1 h before H_3PO_4 (0.645 mL, 85%) was slowly added. The resulting mixture was then stirred at reflux for another 8 h. After removal of the solvent, the dark residue was poured onto ice (10 g) and neutralized with 40% aqueous KOH solution until the solution was at pH = 9. The resulting mixture was extracted with dichloromethane (3x10 mL). The organic phases were combined

and dried ($MgSO_4$). The product was flash chromatographed with ethyl acetate/hexane. Yield: 30% (0.3895 g, 0.0013 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.17 – 8.08 (m, 2H, Ar-*H*), 7.58 (s, 1H, C*H*$_{Im}$), 7.32 – 7.23 (m, 2H, Ar-*H*), 7.16 -7.12 (m, 1H, C*H*$_{Im}$), 6.94 -6.89 (m, 1H, C*H*$_{Im}$), 5.21 (s, 2H, C*H*$_2$), 1.43 (s, 9H, *t*-Bu). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 177.67 (Cq, N*C*O), 173.51 (Cq, N*C*N), 140.02 (Cq), 136.89 (CH, N*C*HN), 129.62 (CH, N*C*HCH), 128.09 (*C*H), 126.99 (*C*H), 123.78 (Cq), 118.60 (CH, CH*C*HN), 49.74 (CH_2), 31.88 (Cq, -*C*(CH_3)$_3$), 27.82 ((*C*H$_3$)$_3$C-). MS: *m*/*z* = 282.2 (M^+, 60), 267.1 (5), 215.2 (100).

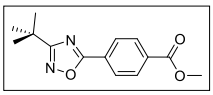
Synthesis of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)benzoic acid (32)



A suspension of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)benzonitrile (10 g, 44.00 mmol) and 8.8 g of NaOH (220.00 mmol) in H_2O (150 mL) was heated to reflux for 3 h. The mixture was cooled to room temperature and the pH was adjusted 2 using 2 N HCl. The solid thus formed was filtered off, washed with plenty of water (100 mL) and dried. Yield*:* 56% (5.59 g, 24.64 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.26 (s, 4H, Ar-*H*), 1.45 (s, 9H, *t*-Bu). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 179.13 (Cq, N*C*O), 165.67 (Cq, *C*OOH), 158.45 (Cq, N*C*N), 131.41 (Cq, *C*COOH), 129.25 (Cq), 128.82 (*C*H), 34.76 (Cq, *C*(CH_3)$_3$), 28.12 ((*C*H$_3$)$_3$C). MS: EI *m*/*z* = 246.1 (M^+, 30), 231.1 (35), 149.1 (100).

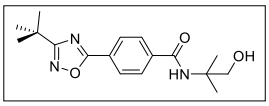
Synthesis of methyl 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)benzoate (33)



A suspension of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)benzoic acid (1.0 g, 4.06 mmol) in 50 mL of diethyl ether was cooled in an ice bath and a 2.5 N solution of diazomethane in diethyl ether was added dropwise until the colorless solid disappeared and the gas evolution stopped. The solvent was removed under high vacuum to afford a colorless solid. No further purification was necessary. Yield*:* 1.02 g (39.43 mmol, 97%). Crystals suitable for X-ray diffraction analysis were formed by slow evaporation of a dichloromethane solution at room temperature.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.27 -8.13 (m, 4H, Ar-*H*), 3.96 (s, 3H, C*H*$_3$), 1.44 (s, 9H, *t*-Bu). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.61 (Cq, NCO), 174.23 (Cq, *C*OOMe), 166.09 (Cq, NCN), 133.45 (Cq, *C*COOMe), 130.14 (*C*H), 128.30 (Cq), 128.05 (*C*H), 52.47 (Cq, *C*H$_3$), 32.57 (Cq, *C*(CH_3)$_3$), 28.47 ((*C*H$_3$)$_3$C). MS: EI *m*/*z* = 260.1 (M^+, 30), 245.1 (30), 163.1 (100).

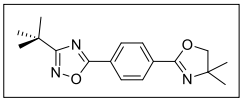
Synthesis of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)-N-(1-hydroxy-2-methylpropan-2-yl)benzamide (34)



A mixture of 4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)benzoic acid (5 g, 20.3 mmol) and 4.42 mL of $SOCl_2$ (7.24 g, 60.91 mmol) was stirred at ambient temperature under an argon atmosphere for 3 h. The excess $SOCl_2$ was removed by distillation and the residue, 4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)benzoyl chloride, was dissolved in DCM (25 mL) and added dropwise to a solution containing 3.61 g of 2-amino-2-methyl-1-propanol (40.6 mmol) in DCM (25 mL) at 0 ºC. After the addition was complete, the reaction was stirred for 12 h. The precipitate was removed by filtration. The filtrate was evaporated and the resulting residue was crystallized from Et_2O. Yield*:* 76% (4.89 g, 15.42 mmol).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.13 – 8.07 (m, 2H, Ar-*H*), 7.82 – 7.77 (m, 2H, Ar-*H*), 6.37 (bs, 1H, N*H*), 4.33 (bs, 1H, O*H*), 3.66 (s, 2H, CH_2), 1.38 (s, 6H, 2 x CH_3), 1.36 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.57 (Cq, N*C*O), 174.09 (Cq, NH*C*O), 167.09 (Cq, N*C*N), 138.26 (Cq, *C*CONH), 128.25 (*C*H), 127.56 (*C*H), 127.13 (Cq, C*C*ONH), 70.38 (Cq), 70.38 (Cq), 56.66 (*C*H_2OH), 32.54 (Cq, *C*$(CH_3)_3$), 28.45 ((*C*H_3)$_3$C), 24.49 ((*C*H_3)$_2$C). MS (ESI): 318.13 (100, M + H^+), 340.0 (60, M + Na^+), 656.8 (55, 2 x M + Na^+).

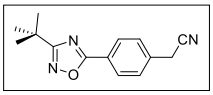
Synthesis of 3-*tert*-butyl-5-(4-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)phenyl)-1,2,4-oxadiazole (35)



15 mL of $SOCl_2$ was added dropwise to 4 g of the amide (12.6 mmol) under argon atmosphere. After the solution was stirred for 2 h, 15 mL of methanol was added to destroy the excess of $SOCl_2$. The reaction mixture was then poured into 100 mL of KOH (4 N) solution and twice extracted with Et_2O (2 × 100 mL). The ethereal layer was separated, dried over Na_2SO_4 and evaporated. Yield*:* 86% (3.24 g, 10.8 mmol). Single crystals suitable for X-ray diffraction analysis were generated by slow diffusion of hexane into a concentrated DCM solution.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.11 – 8.06 (m, 2H, Ar-*H*), 8.02 – 7.97 (m, 2H, Ar-*H*), 4.06 (s, 2H, CH_2), 1.35 (s, 9H, *t*-Bu), 1.32 (s, 6H, 2 x CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.43 (Cq, NCO), 174.41 (Cq, NCO), 161.07 (Cq, NCN), 131.64 (Cq, *C*CON), 128.73 (CH), 127.88 (*C*H), 126.64 (Cq), 79.27 (O*C*H_2C), 67.88 (Cq), 32.48 (Cq, *C*$(CH_3)_3$), 28.46 ((*C*H_3)$_3$C), 28.34((**C**H_3)$_2$C). MS (ESI): 300.17 (100, M + H^+), 301.17 (20, M + H^+).

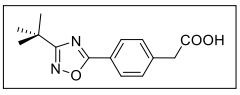
Synthesis of 2-[4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl]acetonitrile (36)



A solution of 4-(cyanomethyl)benzoic acid (27.3 g, 0.17 mol) in DMF (250 mL) was treated with a solution of CDI (30.2 g, 0.187 mol) in DMF (200 mL). After 30 min stirring at room temperature, a solution of *tert*-butylamidoxime (21.7 g, 0.187 mol) in DMF (100 mL) was added, and the reaction mixture was stirred for 1 h at room temperature. A second portion of CDI (27.3 g, 0.17 mol) dissolved in DMF (200 mL) was added and the mixture was heated to reflux for 6 h. The mixture was cooled to room temperature and poured into a water-ice mixture. The solid thus formed was filtered off, washed with water and dried. Yield raw: 70% (17.13 g, 0.12 mol). The substance is approximatively 60% pure according to the spectroscopical data.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.12 – 8.03 (m, 2H, Ar-*H*), 7.78 – 7.62 (m, 2H, Ar-*H*), 3.65 (s, 2H, C*H*$_2$) 1.44 (s, 9H, *t*-Bu). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 179.18 (Cq, N*C*O), 171.46 (Cq, N*C*N), 132.56 (*C*H), 128.38 (*C*H), 127.69 (Cq), 126.89 (Cq), 110.62 (Cq, *C*N), 40.12 (*C*H$_2$), 32.22 (Cq, *C*(CH$_3$)$_3$), 28.23 ((*C*H$_3$)$_3$C).

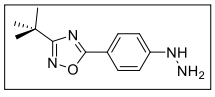
Synthesis of 2-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)acetic acid (37)



A suspension of 15 g 2-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)acetonitrile (62.24 mmol) and 12.44 g of NaOH (311.20 mmol) in H_2O (200 mL) was heated to reflux for 5 h. The mixture was cooled to room temperature and the pH was adjusted to 2 using 2 *N* HCl. The solid thus formed was filtered off, washed with plenty (150 mL) of water and dried. Yield*:* 66% (10.68 g, 41.08 mmol). Single crystals were grown by slow evaporation of a concentrated solution in MeOH/DCM.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.06 – 7.96 (m, 2H, Ar-*H*), 7.41 – 7.29 (m, 2H, Ar-*H*), 3.65 (s, 2H, C*H*$_2$), 1.35 (s, 9H, *t*-Bu). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 179.97 (Cq, *C*OOH), 179.11 (Cq, N*C*O), 158.65 (Cq, N*C*N), 137.37 (Cq), 129.43 (*C*H), 127.73 (*C*H), 123.05 (Cq), 40.32 (*C*H$_2$), 31.87 (Cq, *C*(CH$_3$)$_3$), 27.83 ((CH$_3$)$_3$C). MS: EI *m*/*z* = 260.1 (M^+, 30), 245.1 (35), 163.1 (100).

Synthesis of 3-*tert*-butyl-5-(4-hydrazinylphenyl)-1,2,4-oxadiazole (38)

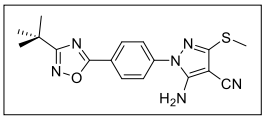


To a solution of 5.0 g 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)aniline (23.02 mmol) in propionic acid (30 mL) cooled at 0ºC was added dropwise to a solution of 1.75 g of $NaNO_2$ (25.32 mmol) in H_2SO_4 (14.7 mL). After stirring the reaction

mixture for 1 h at 0ºC the cooling was removed and the mixture was stirred 1h at room temperature. The reaction was cooled again to 0ºC and a solution of 13.9 g of $SnCl_2$ (73.6) in 37% HCl (12.5 mL) was added dropwise. The cooling was removed and the mixture was stirred 1h at room temperature. Afterwards the reaction was diluted with a solution of NaOH (50%) until pH-14, and the mixture was extracted with Et_2O (3x100 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was chromatographed on a column of silica gel with ethyl acetate-hexane (1: 1). Yield*:* 80% (4.3 g, 18.5 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.94 – 7.84 (m, 2H, Ar-*H*), 6.85 – 6.73 (m, 2H, Ar-*H*), 5.72 – 5.44 (bs, N*H*), 3.71 – 3.48 (bs, NH_2), 1.34 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.05 (Cq, N*C*O), 175.34 (Cq, N*C*N), 154.16 (Cq, *C*NHNH$_2$), 129.67 (*C*H), 114.78 (Cq), 111.32 (*C*H), 32.38 (Cq, *C*(CH$_3$)$_3$), 28.52 ((*C*H$_3$)$_3$C) ppm. MS: *m*/*z* = 232.2 (M^+, 55), 217.2 (15), 145.1 (100).

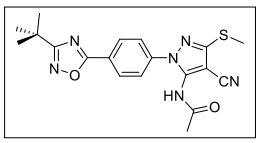
Synthesis of 5-amino-1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(methylthio)-1*H*-pyrazole-4-carbonitrile (39)



A mixture of 3 g 3-*tert*-butyl-5-(4-hydrazinylphenyl)-1,2,4-oxadiazole (12.93 mmol) and 2.20 g (12.93 mmol) of (bis(methylthio)methylene)malononitrile in methanol (50 mL) was refluxed for 6 h. After cooling, the resulting precipitate was collected and crystallized from methanol to afford colorless solid. Yield*:* 76% (3.48 g, 9.8 mmol).

^{1}H NMR (300.1 MHz, DMSO-d$_6$): δ = 8.24 – 8.19 (m, 2H, Ar-*H*), 7.82 – 7.76 (m, 2H, Ar-*H*), 7.19 – 7.06 (bs, NH_2), 2.80 (s, 3H, -CH_3), 1.40 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, DMSO-d$_6$): δ = 177.86 (Cq, N*C*O), 173.95 (Cq, N*C*N), 152.68 (Cq, *C*NH$_2$), 152.68 (Cq, *C*SCH$_3$), 150.11 (Cq), 140.85 (Cq), 129.05 (*C*H), 124.04 (*C*H), 124.04 (Cq, *C*N), 73.94 (Cq, *C*CN), 32.12 (Cq, *C*(CH$_3$)$_3$), 28.12 ((*C*H$_3$)$_3$C), 13.16 (S*C*H$_3$) ppm. MS (HR-ESI): calcd. For $C_{17}H_{19}N_6OS$ + H: 355, 1341; found: 355,1362 (100).

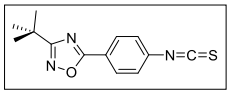
Synthesis of *N*-(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-4-cyano-3-(methylthio)-1H-pyrazol-5-yl)acetamide (40)



A solution of 2 g 5-amino-1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(methylthio)-1*H*-pyrazole-4-carbonitrile (5.64 mmol) in glacial acetic acid (15 mL) and acetic anhydride (20 mL) was heated to reflux for 12 h. The solvent was removed under reduced pressure to give a solid, which was crystallized from methanol to afford colorless crystals. Yield*:* 57% (1.27 g, 3.21 mmol).

^{1}H NMR (300.1 MHz, DMSO-d_6): δ = 10.98 – 10.72 (bs, N*H*), 8.29 – 8.22 (m, 2H, Ar-*H*), 7.84 – 7.72 (m, 2H, Ar-*H*), 2.63 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 1.40 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, DMSO-d_6): δ = 177.90 (Cq, N*C*O), 173.78 (Cq, *C*=O), 168.90 (Cq, N*C*N), 150.87 (Cq, *C*NH), 142.42 (Cq, *C*S), 140.56 (Cq), 129.11 (*C*H), 124.19 (*C*H), 123.20 (Cq), 111.75 (Cq, *C*N), 89.23 (Cq, *C*CN), 32.13 (Cq, *C*(CH_3)$_3$), 28.11 ((*C*H_3)$_3$C), 22.61 (CO*C*H_3), 13.51 (S*C*H_3) ppm. MS (HR-ESI): calcd. For $C_{19}H_{20}N_6O_2S$ + Na: 419,1266; found: 419,1254 (100); (2 X $C_{19}H_{20}N_6O_2S$) + Na: 815,2634; found: 815,2617 (35).

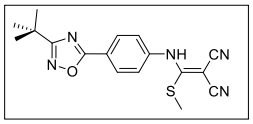
Synthesis of 3-*tert*-butyl-5-(4-isothiocyanatophenyl)-1,2,4-oxadiazole (41)



To a solution of 6.1 g Na_2CO_3 (57.52 mmol) in 30 mL of water (cooled at 0-5ºC) a solution of 7.51 g of triphosgene (25.31 mmol) in DCM (30 mL) was added. The mixture was stirred vigorously for 5 minutes at 0-5ºC. Then 5 g of 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)aniline (23.01 mmol) dissolved in 10 mL of DCM was added slowly and the cooling was removed. The resulting mixture was stirred for 4 hours at room temperature. When the reaction was finished, the two phases were separated. The aqueous phase was washed 2 times with dichloromethane and then all DCM phases were combined and washed with water, dried on Na_2SO_4 and evaporated. Yield*:* 96% (5.73 g, 22.09 mmol).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.12 – 7.97 (m, 2H, Ar-*H*), 7.33 – 7.24 (m, 2H, Ar-*H*), 1.36 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 178.53 (Cq, N*C*O), 173.93 (Cq, N*C*N), 135.24 (Cq, N*C*S), 129.45 (*C*H), 129.36 (Cq), 126.29 (*C*H), 123.15 (Cq), 32.55 (Cq, *C*(CH_3)$_3$), 28.46 ((*C*H_3)$_3$C) ppm. MS: EI *m*/*z* = 259.1 (M^+, 60), 244.1 (15), 162.1 (100).

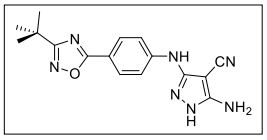
Synthesis of 2-(((4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)amino)(methylthio)-methylene)malononitrile (42)



To an ice cooled solution of 1.32 g malononitrile (20mmol), 5.18 g of 3-*tert*-butyl-5-(4-isothiocyanatophenyl)-1,2,4-oxadiazole (20 mmol) and 2.84 g of iodomethane (20 mmol) in anhydrous DMF (25 mL), was added in a single portion 0.88 g of 60% sodium hydride dispersion in mineral oil (0.88 g, 20 mmol). The resulting mixture was stirred at room temperature for 2 h, then at 50–55 ºC for 30 min. The solid formed after adding water (150 mL) was filtered, dissolved in dichloromethane and dried. Evaporating the solvent in vacuum gave a residue that was crystallized from methanol dichloromethane 4:1 to give a colorless solid. Yield*:* 90% (6.11 g, 18 mmol).

^{1}H NMR (200 MHz, DMSO-d$_6$): δ = 11.01 – 10.73 (bs, N*H*), 8.22 – 8.09 (m, 2H, Ar-*H*), 7.63 – 7.47 (m, 2H, Ar-*H*), 2.59 (s, 3H, -C*H*$_3$), 1.39 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, DMSO-d$_6$): δ = 177.66 (Cq, NH*C*S), 173.95 (Cq, N*C*O), 171.79 (Cq, N*C*N), 142.61 (Cq), 128.69 (*C*H), 123.26 (*C*H), 121.21 (Cq, *C*N), 120.39 (Cq), 55.65 (Cq, *C*(CN)$_2$), 31.98 (Cq, *C*(CH$_3$)$_3$), 28.02 ((*C*H$_3$)$_3$C), 15.87 (S*C*H$_3$) ppm. MS: EI *m*/*z* = 339.1 (M$^+$, 60), 291.1 (50), 194.0 (100).

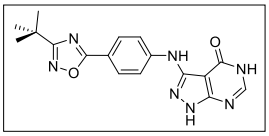
Synthesis of 5-amino-3-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylamino)-1*H*-pyrazole-4-carbonitrile (43)



A mixture of 5 g 2-((4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylamino)-(methylthio)methylene)malononitrile (14.75 mmol) and 0.866 mL of hydrazine monohydrate (0.894, 17.85 mmol) in methanol (20 mL) was stirred for 1 h at 65 °C. After cooling at room temperature, the mixture was diluted with water (50 ml) and stirred for 1 h. The resulting precipitate was collected by filtration and washed with water (50 ml) to give a colorless solid. Yield*:* 93% (4.43 g, 13.71 mmol).

^{1}H NMR (200 MHz, DMSO-d$_6$): δ = 11.46 – 11.32 (bs, N*H*), 9.18 – 9.02 (bs, N*H*), 7.96 – 7.85 (m, 2H, Ar-*H*), 7.67 – 7.56 (m, 2H, Ar-*H*), 6.48 – 6.28 (bs, N*H*$_2$), 1.35 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (50 MHz, DMSO-d$_6$): δ = 177.32 (Cq, N*C*O), 174.75 (Cq, N*C*N), 152.89 (Cq, NH*C*), 149.59 (Cq, *C*NH$_2$), 146.85 (Cq), 128.68 (*C*H), 115.75 (*C*H), 114.74 (Cq), 113.86 (Cq, *C*N), 31.90 (Cq, *C*(CH$_3$)$_3$), 28.11 ((*C*H$_3$)$_3$C) ppm. MS (HR-ESI): calcd. for C$_{16}$H$_{17}$N$_7$O + H: 324,1572; found: 324,1568 (15); C$_{16}$H$_{17}$N$_7$O + Na: 346,1392; found: 346,1388 (100).

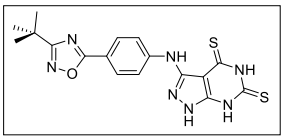
Synthesis of 3-((4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)amino)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (44)



A mixture of 1.0 g 5-amino-3-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylamino)-1*H*-pyrazole-4-carbonitrile (3.09 mmol) and formic acid (35 mL) was refluxed for 2 h. The reaction mixture was cooled to room temperature, and then added slowly to ice water. The solid thus formed was collected by filtration, washed with water and dried. The solid was chromatographed on a column of silica gel with ethyl acetate-hexane (1: 2). Yield*:* 63% (0.685 g, 1.95 mmol).

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 13.27 – 12.92 (bs, N*H*), 12.24 – 11.96 (bs, N*H*), 8.74 – 8.65 (bs, N*H*), 8.02 (s, 1H, -C*H*$_{\text{Pyrimidine}}$), 7.98 – 7.94 (m, 2H, Ar-*H*), 7.87 – 7.81 (m, 2H, Ar-*H*), 1.36 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = (DMSO-d$_6$, 100 MHz): 177.45 (Cq, N*C*O), 174.80 (Cq, *C*=O), 157.75 (Cq, N*C*N), 152.80 (Cq, NH*C*N), 148,67 (Cq, NH*C*N), 146,16 (NH*C*HN), 146,05 (Cq), 128.79 (*C*H), 116.37 (*C*H), 114.24 (Cq), 113.03 (Cq), 32.01 (Cq, *C*(CH$_3$)$_3$), 28.19 ((*C*H$_3$)$_3$C) ppm. MS (ESI): 352.15 (20, M + H$^+$), 374.13 (100, M + Na$^+$).

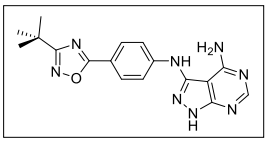
Synthesis of 3-((4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)amino)-1,7-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*)-dithione (45)



A mixture of 1.0 g 5-amino-3-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylamino)-1*H*-pyrazole-4-carbonitrile (3.09 mmol) and CS_2 (5 mL) in pyridine (25 mL) was refluxed for 24 h. The reaction mixture was cooled to room temperature and the solid thus formed was collected by filtration and recrystallized from DMF. Yield*:* 16% (0.197 g, 0.494 mmol).

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 13.35 – 12.70 (bs, N*H*), 9.64 – 9.53 (bs, 2H, 2 x N*H*), 8.07 – 7.99 (m, 2H, Ar-*H*), 7.80 – 7.69 (m, 2H, Ar-*H*), 3.69 – 3.09 (-bs, N*H*), 1.35 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 179.71 (Cq, *C*=S), 177.54 (Cq, N*C*O), 174.56 (Cq, *C*=S), 172.34 (Cq, N*C*N), 157,58 (Cq, NH*C*N), 147,37 (Cq, NH*C*N), 143,92 (Cq), 129.21 (*C*H), 116.97 (*C*H), 115.59 (Cq), 101.23 (Cq), 32.05 (Cq, *C*(CH$_3$)$_3$), 28.20 ((*C*H$_3$)$_3$C) ppm. MS (ESI): 400.10 (100, M + H$^+$).

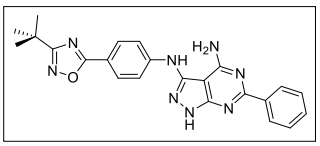
Synthesis of N^3-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamine (46)



A mixture of 1.0 g 5-amino-3-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenylamino)-1*H*-pyrazole-4-carbonitrile (3.09 mmol) and formamide (35 mL) was refluxed for 5 h. The reaction mixture was cooled to room temperature, and then slowly added into ice water. The solid thus formed was collected by filtration, washed with water and cold ethanol and dried. The solid was chromatographed on a column of silica gel with ethyl acetate-hexane (1: 1). Yield*:* 51% (0.552 g, 1.57 mmol).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.83 – 12.74 (bs, N*H*), 9.02 – 8.98 (bs, N*H*), 8.12 (s, 1H, -C$H_{\text{Pyrimidine}}$), 8.03 – 7.97 (m, 2H, Ar-*H*), 7.83 – 7.77 (m, 2H, Ar-*H*), 7.56 – 7.48 (bs, 2H, NH_2), 1.33 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 177.46 (Cq, N*C*O), 170.28 (Cq, N*C*N), 157.35 (Cq, *C*NH_2), 156,28 (Cq, NH*C*N), 154,88 (CH, NH*C*HN), 146,87 (Cq, NH*C*N), 142,18 (Cq), 128.90 (*C*H), 116.26 (*C*H), 114.16 (Cq), 90.88 (Cq), 32.01 (Cq, *C*(CH_3)$_3$), 28.18 ((*C*H_3)$_3$C) ppm. MS (HR-ESI): calcd. for $C_{17}H_{18}N_8O$+ H: 351,1681; found: 351,1676 (100).

Synthesis of N^3-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-6-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamine (47)

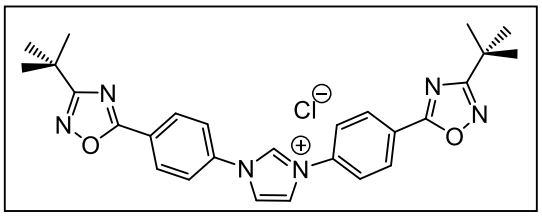


A mixture of 1.0 g 5-amino-3-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-*yl*)phenylamino)-1*H*-pyrazole-4-carbonitrile (3.09 mmol), 0.726 g benzamidine hydrochloride hydrate (4.63 mmol) and 0.507 g of sodium acetate (6.18 mmol) was heated for 30 minutes at 220ºC in naphthalene (20 g). The reaction mixture was cooled to room temperature, and then slowly added into ice water. The solid thus formed was collected by filtration, washed with water and cold ethanol and dried. The solid was chromatographed on a column of silica gel with chloroform-methanol (10: 1). Yield*:* 23% (0.303 g, 0.710 mmol).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.91 – 12.82 (bs, N*H*), 9.07 – 8.98 (bs, N*H*), 8.43 – 8.34 (m, 2H, Ar-*H*), 8.05 – 7.98 (m, 2H, Ar-*H*), 7.87 – 7.82 (m, 2H, Ar-*H*), 7.69 – 7.56 (bs, 2H, NH_2), 7.53 – 7.44 (m, 3H, Ar-*H*), 1.36 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 177.51 (Cq, N*C*O), 174.85 (Cq, Bz*C*N_2), 161.37 (Cq, N*C*N), 157.35 (Cq, N*C*NH_2), 156.29 (Cq, NH*C*N), 146.90 (Cq, NH*C*N), 142,36 (Cq), 138.15 (Cq), 130.10 (Cq), 128.96

(*C*H), 128.21 (*C*H), 127.89 (*C*H), 116.34 (*C*H), 114.19 (Cq), 89.78 (Cq), 32.05 (Cq, *C*$(CH_3)_3$), 28.23 ($(CH_3)_3$C) ppm. MS (HR-ESI): calcd. for $C_{23}H_{22}N_8O$+ H: 427,1994; found: 427,1987 (100); $C_{23}H_{22}N_8O$+ Na: 449,1814; found: 449,1805 (30).

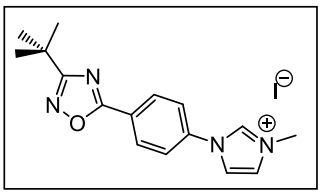
1,3-bis(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-imidazolium chloride (48)



To a solution of paraformaldehyde (0.98 g, 33 mmol) in toluene (40 mL) was added 4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)aniline (14.16 g, 65.2 mmol) and 40% (v/v) aqueous glyoxal (4.74 g, 32.6 mmol) followed by the dropwise addition of 37% (v/v) HCl (3.72 mL, 32.6 mmol). The mixture was heated to reflux and the water was removed using a Dean-Stark apparatus. The solvent was removed under reduced pressure, and the resulting residue was triturated with acetone, leaving a pale-brown solid. Yield*:* 55% (10.5 g, 20.7 mmol).

IR (ATR): 1/λ = 3415, 3360, 3063, 2970, 2931, 2908, 2872, 1610, 1584, 1553, 1524, 1510, 1495, 1467, 1435, 1399, 1353, 1340, 1303, 1270, 1219, 1194, 1106, 1083, 1023, 1006, 987, 952, 909, 854, 840, 773, 735, 696 cm^{-1}. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ = 10.76 (s, 1 H, NC*H*N), 8.78 (br s, 2 H, NC*H*C*H*N), 8.49-8.41 (m, 4 H, Ar-*H*), 8.31-8.22 (m, 4 H, Ar-*H*), 1.42 (s, 18 H, *t-Bu*) ppm. MS (HR-ESI): calcd. for $C_{27}H_{29}N_6O_2^+$: 469.2352; found: 469.2357 (100).

1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-methyl-imidazolium iodide (49)

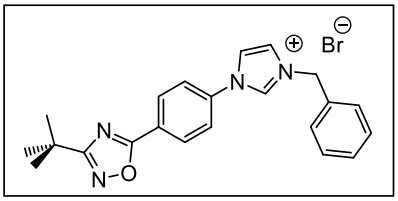


A solution of 5-(4-(1*H*-imidazol-1-yl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (2.5 g, 9.31 mmol) and CH_3I (1.45 g, 0.639 mL, 10.24 mmol) in dry THF (20 mL) was placed under an argon atmosphere and stirred at room temperature for 24 h. The precipitate was collected by filtration, washed with diethyl ether and dried in vacuo, giving a colorless solid. Yield*:* 98% (3.6 g, 9.12 mmol).

IR (ATR): 1/λ = 3152, 3106, 3077, 3053, 3024, 2972, 2929, 2869, 1926, 1700, 1675, 1616, 1594, 1582, 1547, 1522, 1507, 1470, 1441, 1414, 1392, 1356, 1319, 1291, 1241, 1200, 1139, 1099, 1066, 1029, 991, 972, 954, 898, 847, 772, 733, 692, 679, 632, 620 cm^{-1}. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ = 9.98 (s, 1 H, NC*H*N), 8.45 (br s, 1 H, NCH*CH*N), 8.41-8.33 (m, 2 H, Ar-*H*), 8.13-8.01 (m, 3 H, Ar-*H*, N*CH*CHN), 3.99 (s, 3 H, C*H*$_3$), 1.39 (s, 9 H, *t-Bu*) ppm. ^{13}C NMR (75.47 MHz, DMSO-d_6): δ = 177.9 (N*C*O), 173.5 (N*C*N$_{ox}$), 137.3 (N*C*HN$_{Im}$), 136.4

(N*C*$_{Ar}$), 129.7 (*C*H$_{Ar}$), 124.7 (*C*$_{Ar}$), 124.3 (*C*HNCH$_{3Im}$), 122.5 (*C*H$_{Ar}$), 120.6 (*C*HNAr$_{Im}$), 36.3 (*C*H$_3$), 32.1 (*C*CH$_3$), 28.0 (C*C*H$_3$) ppm. MS (HR-ESI): calcd. for $C_{16}H_{19}N_4O^+$: 283.1559; found: 283.1554 (100).

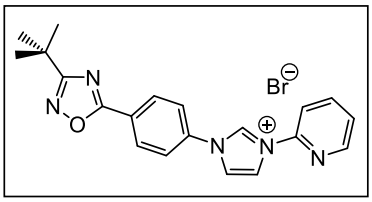
1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(benzyl)-imidazolium bromide (50)



A solution of 5-(4-(1*H*-imidazol-1-yl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (0.5 g, 1.86 mmol) and 0.22 mL of benzyl bromide (0.318 g, 1.86 mmol) in dry Toluene (10 mL) was placed under an inert atmosphere and stirred for 48 h. After cooling, the imidazolium salt was precipitated by adding Et_2O (50 mL). After filtration and drying under vacuum, a colorless solid was obtained. Yield*:* 84% (0.685 g, 1.56 mmol).

IR (ATR): 1/λ = 3357, 3153, 3114, 3030, 2973, 2930, 2904, 2870, 1614, 1566, 1545, 1521, 1507, 1497, 1468, 1452, 1434, 1419, 1396, 1371, 1352, 1287, 1236, 1224, 1194, 1100, 1066, 1028, 954, 858, 846, 827, 813, 772, 759, 735, 716, 691, 691, 626 cm^{-1}. ^{1}H NMR (300.1 MHz, DMSO-d$_6$): δ = 10.41 (s, 1 H, NC*H*N), 8.53 (s, 1 H, C*H$_{Im}$*), 8.42-8.33 (m, 2 H, Ar-*H*), 8.22-8.15 (m, 2 H, Ar-*H*), 8.12 (s, 1 H, C*H$_{Im}$*), 7.67-7.58 (m, 2 H, Ar-*H$_{Bn}$*), 7.52-7.38 (m, 3 H, Ar-*H$_{Bn}$*), 5.61 (s, 2 H, C*H$_2$*), 1.39 (s, 9 H, *t-Bu*) ppm. ^{13}C NMR (75.47 MHz, DMSO-d$_6$): δ = 177.9 (N*C*O), 173.5 (N*C*N$_{ox}$), 137.8 (N*CH*N$_{Im}$), 136.0, 134.3 (N*C$_{Ar}$*), 129.6, 128.9, 128.8, 128.6, 124.3, 123.4 (*C*H$_{Im}$), 122.7, 121.3 (*C*H$_{Im}$), 52.4 (*C*H$_2$-Bn), 32.1 (*C*CH*3*), 28.1 (C*C*H$_3$) ppm. MS (HR-ESI): calculated for $C_{22}H_{23}ON_4^+$: 359.1866 (100), 360.1900 (25), 361.1932 (5); found: 359.1856 (100), 360.1885 (25), 361.1919 (5).

1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2-pyridine)-imidazolium bromide (51)

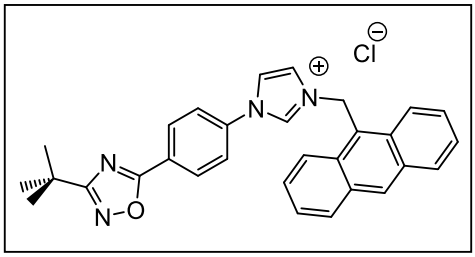


A solution of 5-(4-(1*H*-imidazol-1-yl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (0.3 g, 1.12 mmol) and 2-bromopyridine (3.314 g, 2 mL, 20.97 mmol) was placed under an argon atmosphere and stirred at 160 °C for 48 h. The reaction was cooled to room temperature and Et_2O was added. The precipitate thus formed was collected by filtration, washed with diethyl ether and dried in vacuum. It remained a light grey solid. Yield*:* 93% (0.36 g, 1.04 mmol).

IR (ATR): 1/λ = 3538, 3176, 3097, 3060, 3042, 2974, 2931, 2903, 2869, 2819, 2359, 2339, 1805, 1727, 1646, 1617, 1598, 1573, 1548, 1523, 1509, 1476, 1443, 1445, 1395, 1351, 1322, 1290, 1271, 1198, 1148, 1102, 1064, 1017, 992, 971, 950, 882, 850, 830, 785, 772, 735, 711,

694, 639, 617 cm^{-1}. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ = 10.88 (s, 1 H, NC*H*N), 8.83 (m, 1 H, CH_{Py}), 8.77 (m, 1 H, CH_{Py}), 8.73 (d, 1 H, CH_{Py}), 8.43-8.38 (m, 2 H, Ar-*H*), 8.36-8.27 (m, 4 H, CH_{Im}, Ar-*H*), 7.77-7,69 (m, 1 H, CH_{Py}), 1.41 (s, 9 H, *t-Bu*) ppm. ^{13}C NMR (75.47 MHz, DMSO-d_6): δ = 177.9 (N*C*O), 173.5 (N*C*N_{ox}), 149.2 (C_{Py}), 146.1 (CH_{Py}), 140.6 (CH_{Py}), 137.6 (N*C*HN_{Im}), 134.8 (NC_{Ar}), 129.5 (CH_{Ar}), 125.6 (C_{Ar}), 124.7 (CH_{Py}), 123.1 (CH_{Ar}), 121.9 (CH_{Im}), 120.1 (CH_{Im}), 114.9 (CH_{Py}), 32.2 (*C*CH_3), 28.1 (C*C*H_3) ppm. MS (HR-ESI): calcd. for $C_{20}H_{20}AuN_5O^+$: 346.1668 (100), 347.1701 (20), 348.1735 (3); found: 346.1663 (100), 347.1704 (20), 348.1731 (3).

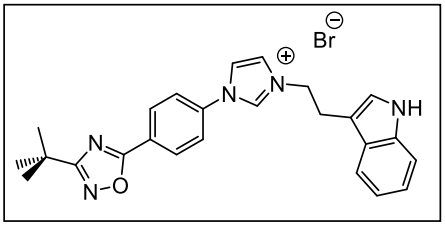
1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(9-methyl-anthracene)-imidazolium chloride (52)



A solution of 5-(4-(1*H*-imidazol-1-yl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (1 g, 3.72 mmol) and 9-(chloromethyl)anthracene (0.85 g, 3.72 mmol) in dry toluene (50 mL) was placed under an inert atmosphere and stirred under reflux for 48 h. The precipitate was collected by filtration, washed with diethyl ether and dried in vacuum, giving a gray solid. Yield*:* 24% (0.45 g, 0.9 mmol).

IR (ATR): 1/λ = 3167, 3051, 3024, 2974, 2958, 2933, 2905, 2846, 2822, 1949, 1928, 1617, 1596, 1552, 1523, 1508, 1476, 1465, 1451, 1439, 1424, 1395, 1373, 1356, 1314, 1291, 1235, 1198, 1159, 1144, 1112, 1102, 1071, 1055, 1018, 993, 953, 886, 868, 854, 844, 827, 796, 774, 736, 721, 697, 624 cm^{-1}. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ = 10.49 (s, 1 H, NC*H*N), 8.86 (s, 1 H, CH_{An}), 8.62-8.52 (m, 2 H, Ar-*H*), 8.44 (s, 1 H, NCHC*H*N), 8.36-8.15 (m, 4 H, CH_{An}), 8.12-7.98 (m, 2 H, Ar-*H*), 7.93-7.44 (m, 5 H, CH_{An}, NC*H*CHN), 6.67 (s, 2 H, CH_2), 1.38 (s, 9 H, *t-Bu*) ppm. ^{13}C NMR (75.47 MHz, DMSO-d_6): δ = 177.9 (N*C*O), 173.4 (N*C*N_{ox}), 147.2 (N*C*HN_{Im}), 135.8 (NC_{Ar}), 131.0, 130.8, 130.3, 129.5, 129.3, 127.8, 125.5, 124.3 (CH_{Im}), 123.6, 123.1, 122.7, 121.2 (CH_{Im}), 45.5 (CH_2-An), 32.1 (*C*CH_3), 28.1 (C*C*H_3) ppm. MS (HR-ESI): calcd. for $C_{30}H_{27}N_4O^+$: 459.2185; found: 459.2185 (100).

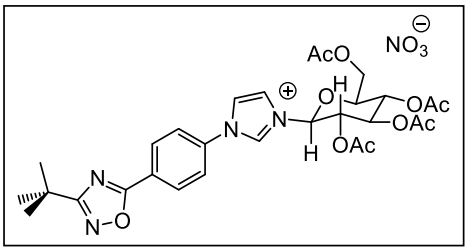
1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-((2-ethyl)indole)-imidazolium bromide (53)



A solution of 5-(4-(1*H*-imidazol-1-yl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (0.5 g, 1.86 mmol) and 3-(2-bromoethyl)indole (0.418 g, 1.86 mmol) in dry toluene (30 mL) was placed under an inert atmosphere and stirred under reflux for 48 h. The precipitate was collected by filtration, washed with diethyl ether, dried and flash chromatographed with DCM/MeOH. Yield*:* 20% (0.180 g, 0.365 mmol).

IR (ATR): 1/λ = 3357, 3129, 3063, 2974, 2972, 2869, 1616, 1542, 1475, 1429, 1403, 1355, 1281, 1228, 1196, 1097, 1011, 963, 854, 783, 743, 699, 685, 643, 608, 591, 558. ^{1}H NMR (300.1 MHz, DMSO-d$_6$): δ = 11.04 (s, 1 H, N*H*), 10.09 (s, 1 H, NC*H*N), 8.47 (s, 1 H, CH_{In}), 8.42-8.31 (m, 2 H, Ar-*H*), 8.14 (s, 1 H, CH_{Im}), 8.08-7.97 (m, 2 H, Ar-*H*), 7.66-7.58 (m, 1 H, CH_{In}), 7.44-7.35 (m, 1 H, CH_{In}), 7.26 (s, 1 H, CH_{Im}), 7.14-7.06 (m, 1 H, CH_{In}), 7.05-6.94 (m, 1 H, CH_{In}), 4.68-4.53 (m, 2 H, CH_2), 3.47-3.38 (m, 2 H, CH_2), 1.41 (s, 9 H, *t-Bu*) ppm. ^{13}C NMR (75.47 MHz, DMSO-d$_6$): δ = 177.9 (N*C*O), 173.5 (N*C*N$_{ox}$), 138.4 (N*C*HN$_{Im}$), 137.7, 136.1, 135.8, 135.3, 129.6, 129.5, 126.8, 124.3, 123.8, 123.5, 123.2, 122.5, 122.3, 121.2, 120.6, 119.96, 118.5, 117.9, 111.5 (Ar-*C*H$_{In}$), 109.1 (CH$_2$-*C*$_{In}$), 50.2 (*C*H$_2$), 32.2 (*C*CH$_3$), 28.1 (C*C*H$_3$), 25.6 (*C*H$_2$) ppm. MS (HR-ESI): calculated for $C_{25}H_{26}N_5O^+$: 412.2137; found: 412.2135.

1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2,3,4,5-tetra-*O*-acetyl-*D*-glucopyranosyl)-imidazolium nitrate (54)

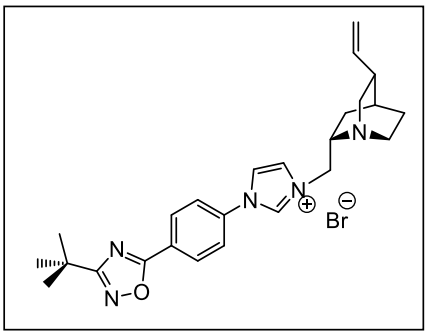


To a solution of 2,3,4,5-tetra-*O*-acetyl-*D*-glucopyranosylbromide (1.68 g, 4.09 mmol) in acetonitrile (20 ml) were added silver nitrate (0.7 g, 4.09 mmol) and 5-(4-(1*H*-imidazol-1-yl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (1 g, 3.72 mmol). The reaction mixture was stirred at 50° C for 24 h. All insoluble materials were removed by filtration through Celite, and the solution was concentrated under reduced pressure. The resulting solid was dissolved in DCM and stirred over Na_2CO_3 for 30 min. After filtration the product was recrystallized several times from DCM / Methyl-*tert*-butylether to give a

colorless solid. Only the β isomer was observed in NMR spectra. Yield*:* 18% (0.45 g, 0.68 mmol).

IR (ATR): 1/λ = 3098, 2972, 2933, 2360, 2340, 1754, 1747, 1618, 1552, 1510, 1432, 1396, 1368, 1350, 1331, 1314, 1244, 1219, 1195, 1162, 1122, 1090, 1068, 1052, 1017, 954, 943, 919, 896, 852, 828, 775, 737, 696, 652, 625 cm^{-1}. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 11.09 (s, 1 H, NC*H*N), 8.39-8.31 (m, 2 H, Ar-*H*), 8.12 (s, 1 H, CH_{Im}), 8.02-7.94 (m, 2 H, Ar-*H*), 7.91 (s, 1 H, CH_{Im}), 6.46 (d, J_{HH} = 8.6 Hz, 1 H, N-C*H*-O), 5.58 (d, J_{HH} = 2.5 Hz, 1 H, C*H2*), 5.51-5.34 (m, 2 H, 2xC$H_{carbohydrate}$), 4.49 (t, J_{HH} = 12.6 Hz, 1 H, C$H_{carbohydrate}$), 4.29-4.15 (m, 1 H, C$H_{carbohydrate}$), 4.21 (d, J_{HH} = 2.3 Hz, 1 H, CH_2), 2.23 (s, 3 H, CH_3), 2.06 (s, 3 H, CH_3), 2.05 (s, 3 H, CH_3), 1.99 (s, 3 H, CH_3), 1.43 (s, 9 H, *t-Bu*) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 178.6 (N*C*O), 173.1 (N*C*N_{ox}), 137.8 (N*C*HN_{Im}), 170.4 (CH_3-*C*=O), 169.8 (CH_3-*C*=O), 169.3 (CH_3-*C*=O), 137.3 (N-*C*H-N), 136.8 (*C*$_{Ar}$), 130.3 (*C*H$_{Ar}$), 126.6 (*C*H$_{Im}$), 122.4 (*C*H$_{Ar}$), 121.5 (*C*$_{Ar}$), 120.9 (*C*H$_{Im}$), 85.2 (N-*C*H$_{carbohydrate}$-O), 74.3 (O-*C*H$_{carbohydrate}$-CH_2), 70.3 (*C*H$_{carbohydrate}$), 68.2 (*C*H$_{carbohydrate}$), 66.8 (*C*H$_{carbohydrate}$), 61.1 (CH-*C*H$_{2carbohydrate}$-OAc), 32.5 (*C*-CH_3), 28.4 (C-*C*H_3), 20.6 (COO-*C*H_3), 20.5 (COO-*C*H_3), 20.3 (COO-*C*H_3) ppm. MS (HR-ESI): calculated for $C_{29}H_{35}O_{10}N_4^+$: 599.2347 (100), 600.2378 (30), 601.2404 (10); found: 599.2348 (100), 600.2375 (30), 601.2400 (10).

1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCI)-imidazolium bromide (55)

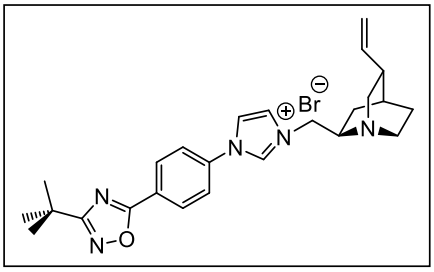


A solution of 5-(4-(1*H*-imidazol-1-yl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (0.3 g, 1.11 mmol) and 0.22 mL of QCI-C_9-Br (0.257 g, 1.11 mmol) in dry toluene (10 mL) was placed under an inert atmosphere and refluxed for 12 h. After cooling, the imidazolium salt was precipitated by adding Et_2O (50 mL). After filtration and drying under vacuum, a colorless solid was obtained. Yield*:* 58% (0.32 g, 0.643 mmol).

IR (ATR): 1/λ = 3162, 3121, 3083, 2957, 2945, 2875, 2865, 1963, 1932, 1829, 1734, 1689, 1666, 1639, 1622, 1597, 1589, 1571, 1543, 1514, 1499, 1465, 1456, 1425, 1407, 1395, 1369, 1349, 1328, 1290, 1275, 1260, 1245, 1198, 1175, 1139, 1124, 1112, 1061, 1053, 1037, 989, 958, 941, 912, 893, 876, 853, 832, 795, 778, 747, 726, 712, 678, 668, 639, 619 cm^{-1}. ^{1}H NMR (300.1 MHz, CD_3OD): δ = 9.26 (bs, 1 H, NC*H*N), 8.42-8.38 (m, 2 H, Ar-*H*), 8.28 (d, J_{HH} = 2.1 Hz, 1 H, CH_{Im}), 8.17 (d, J_{HH} = 2.1 Hz, 1 H, CH_{Im}), 8.12-8.09 (m, 2 H, Ar-*H*), 6.06 (ddd, J_{HH} = 17.49, J_{HH} = 10.01, J_{HH} = 7.50, 1 H, H-10), 5.28-5.13 (m, 2 H, H-11, H-11), 4.17 (dd, *J* = 15.34, *J* = 5.82, 1 H, H-9), 3.67 (dd, *J* = 15.4, *J* = 6.12, 1 H, H-9), 3.53-3.44 (m, 1 H; H-2),

3.19-3.07 (m, 2 H; H-6, H-7), 2.79-2.71 (m, 1 H, H-6), 2.38-2.39 (m, 1 H, H-7), 2.09-1.91 (m,3 H, H-5, H-3, H-4), 1.45 (s, 9 H, *t-Bu*), 1.41-1.38 (m, 2 H, H-8, H-8), 0.93-0.74 (m, 1 H, H-3) ppm. ^{13}C NMR (75.47 MHz, CD_3OD): δ = 179.8 (N*C*O), 175.1 (N*C*N$_{ox}$), 140.3 (*C*H, C-10), 139.3 (N*C*HN$_{Im}$), 130.9 (N*C*$_{Ar}$), 126.9, 125.0 (*C*H$_{Im}$), 124.5, 123.0 (*C*H$_{Im}$), 116.8 (*C*H$_2$, C-11), 56.8 (*C*H$_2$, C-9), 55.3 (*C*H, C-2), 51.4 (*C*H$_2$, C-7), 42.2 (*C*H$_2$, C-6), 39.1 (*C*H, C-4), 33.5 (*C*CH$_3$), 28.8 (C*C*H$_3$), 28.2 (*C*H$_2$, C-8), 26.0 (*C*H, C-5), 25.5 (*C*H$_2$, C-3) ppm. MS (HR-ESI): calculated for $C_{25}H_{32}N_5O^+$: 418.2607 (100), 419.2640 (35); found: 418.2588 (100), 419.2583 (35).

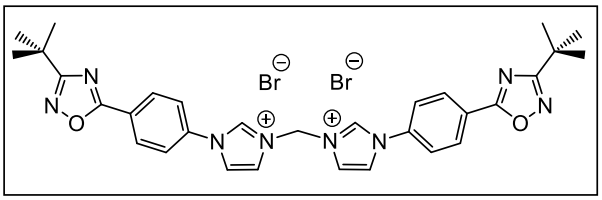
1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCD)-imidazolium bromide (56)



A solution of 5-(4-(1*H*-imidazol-1-yl)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (0.3 g, 1.11 mmol) and 0.22 mL of QCD-C$_9$-Br (0.257 g, 1.11 mmol) in dry toluene (10 mL) was placed under an inert atmosphere and refluxed for 12 h. After cooling, the imidazolium salt was precipitated by adding Et_2O (50 mL). After filtration and drying under vacuum, a colorless solid was obtained. Yield*:* 63% (0.348 g, 0.699 mmol).

IR (ATR): 1/λ = 3125, 3099, 2979, 2949, 2907, 2875, 2859, 1957, 1912, 1731, 1699, 1639, 1619, 1599, 1578, 1551, 1518, 1499, 1468, 1459, 1431, 1396, 1349, 1325, 1311, 1247, 1234, 1199, 1129, 1093, 1063, 1032, 988, 959, 917, 897, 849, 799, 782, 759, 739, 729, 699, 677, 643, 625 cm^{-1}. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 10.96 (s, 1 H, NC*H*N), 8.68 (s, 1 H, C*H*$_{Im}$), 8.27-8.18 (m, 3 H, Ar-*H*, C*H*$_{Im}$), 8.11-8.02 (m, 2 H, Ar-*H*), 6.02-5.91 (m, 1 H, *H-10*), 5.62-5.45 (m, 1 H, H-9), 5.39-5.11 (m, 2 H, H-11, H-11), 4.92-4.73 (m, 1 H, H-9), 3.88-3.71 (m, 1 H; H-2), 3.62-3.36 (m, 2 H; H-6, H-6), 3.31-3.01 (m, 1 H, H-7), 2.76-2.63 (m, 1 H, H-7), 2.31-1.68 (m, 5 H, H-5, H-3, H-4, H-8, H-8), 1.41 (s, 9 H, *t-Bu*), 0.95-0.81 (m, 1 H, H-3) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 178.3 (N*C*O), 173.1 (N*C*N$_{ox}$), 137.2 (CH, C-10), 136.6 (N*C*HN$_{Im}$), 136.1 (N*C*$_{Ar}$), 129.7, 125.5, 124.3 (*C*H$_{Im}$), 122.7, 121.4 (*C*H$_{Im}$), 117.6 (*C*H$_2$, C-11), 57.2 (*C*H, C-2), 48.4 (*C*H$_2$, C-9), 46.1 (*C*H$_2$, C-7), 37.2 (*C*H$_2$, C-6), 40.1 (*C*H, C-4), 32.3 (*C*CH$_3$), 28.2 (C*C*H$_3$), 27.0 (*C*H, C-5), 24.2 (*C*H$_2$, C-8), 23.3 (*C*H$_2$, C-3) ppm. MS (HR-ESI): calculated for $C_{25}H_{32}N_5O^+$: 418.2607 (100), 419.2640 (35); found: 418.2584 (100), 419.2593 (35).

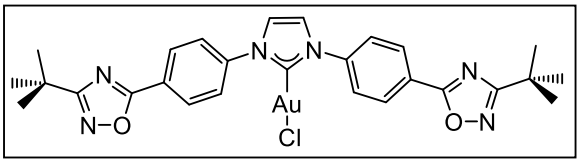
1,1′-[4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl]-3,3′-methylenediimidazolium bis-(bromide) (57)



A mixture of 5-(4-(1*H*-imidazol-1-*yl*)phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole (3 g, 11.13 mmol) and CH_2Br_2 (7.9 mL) was placed under an argon atmosphere and stirred at room temperature for 24 h. The precipitate thus formed was collected by filtration, washed with THF and dried in vacuo, giving a colorless solid. Yield*:* 78% (3.08 g, 8.68 mmol).

IR (ATR): 1/λ = 3121, 3048, 2969, 2957, 2929, 2902, 2870, 2360, 2340, 1767, 1618, 1555, 1520, 1506, 1493, 1469, 1462, 1441, 1395, 1351, 1328, 1286, 1223, 1196, 1099, 1073, 1024, 990, 957, 891, 837, 789, 773, 761, 745, 733, 719, 690, 624 cm^{-1}. ^1H NMR (200.1 MHz, DMSO-d_6): δ = 10.56 (s, 2 H, NC*H*N), 8.62 (s, 2 H, NCHC*H*N), 8.51 (NC*H*CHN), 8.47-8.36 (m, 4 H, Ar-*H*), 8.19-8.06 (m, 4 H, Ar-*H*), 7.02 (s, 2 H, CH_2), 1.41 (s, 18 H, *t-Bu*) ppm. MS (HR-ESI): calculated for $C_{31}H_{34}N_8O_2^{2+}$: 549.2716 (100), 550.2776 (35), 551.2735 (10); found: 549.2623 (100), 550.2756 (35), 551.2795 (10).

Chloro(1,3-bis(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-1*H*-imidazolin-2(3*H*)-ylidene) gold(I) (58)

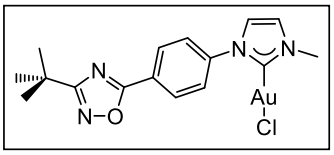


A mixture of 1,3-bis(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)imidazolium chloride (100 mg, 0.198 mmol) and silver(I) oxide (27.5 mg, 0.118 mmol) in dry dichloromethane (15 mL) was stirred for 24 h at room temperature in the dark. The mixture was filtered over Celite, and (dimethyl sulfide)chlorogold(I) (58.32 mg, 0.198 mmol) was added. The resulting mixture was stirred for 12 h and filtered over Celite. The solvent was removed in vacuo, and dichloromethane (2 mL) was added. The product was precipitated as a colorless solid upon addition of pentane and purified by column chromatography (DCM/Ethyl Acetate, 3:1). Yield*:* 59% (83 mg, 0.118 mmol). Single crystals suitable for X-ray diffraction were obtained by slow diffusion of hexane into a concentrated solution of **58** in dichloromethane.

IR (ATR): 1/λ = 3103, 3047, 2967, 2963, 2925, 2852, 2360, 2340, 1733, 1719, 1614, 1593, 1565, 1523, 1511, 1495, 1463, 1436, 1426, 1393, 1351, 1316, 1287, 1260, 1222, 1198, 1096, 1073, 1032, 1017, 992, 972, 943, 884, 849, 803, 775, 745, 737, 697, 679, 629 cm^{-1}. ^1H NMR

(300.1 MHz, $CDCl_3$): δ = 8.38-8.29 (m, 4 H, Ar-*H*), 7.99-7.91 (m, 4 H, Ar-*H*), 7.52 (s, 2 H, *CH*), 1.45 (s, 18 H, *t-Bu*) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 178.7 (N*C*O), 173.6 (N*C*N_{ox}), 170.9 (Au*C*N) 141.7 (N*C*$_{Ar}$), 129.7, 125.8 (*C*H$_{Im}$), 125.4, 122.3 (*C*H$_{Im}$), 32.6 (*C*CH_3), 28.4 (C*C*H_3) ppm. MS (EI): 700.1 (100, M^+), 665.2 (80), 609.1 (70), 540.1 (35) MS (HR-ESI): calcd. for $C_{27}H_{28}AuClN_6O_2Na^+$ (M + Na^+) 723.1525; found 723.1523. Anal. Calcd for $C_{27}H_{28}AuClN_6O_2.H_2O$: C, 45.10; H, 4.21; N, 11.69. Found: C, 44.50; H, 3.57; N, 12.27.

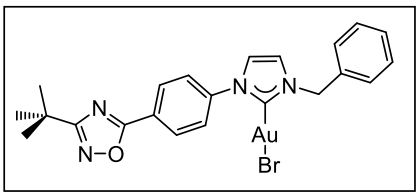
Chloro(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-methyl-1*H*-imidazolin-2(3*H*)-ylidene) gold(I) (59)



A mixture of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-methyl-imidazolium iodide (100 mg, 0.24 mmol) and silver(I) oxide (35 mg, 0.15 mmol) in dry dichloromethane (15 mL) was stirred for 24 h at room temperature in the dark. The mixture was filtered over Celite, and (dimethyl sulfide)chlorogold(I) (71 mg, 0.24 mmol) was added. The resulting mixture was stirred for 12 h and filtered over Celite. The solvent was removed in vacuo, and dichloromethane (2 mL) was added. The product was precipitated as a colorless solid upon addition of pentane and purified by column chromatography (DCM/ethyl acetate, 3:1). Yield*:* 52% (64 mg, 0.124 mmol). Single crystals suitable for X-ray diffraction were obtained by slow evaporation of a concentrated solution of **59** in dichloromethane.

IR (ATR): 1/λ = 3116, 3097, 3067, 2965, 2927, 2871, 1616, 1593, 1519, 1463, 1424, 1410, 1391, 1347, 1319, 1282, 1244, 1193, 1138, 1091, 1032, 986, 970, 956, 890, 854, 775, 743, 696, 673, 632, 602, 551 cm^{-1}. UV/Vis (DCM): λmax. (lg ε) = 223 (1.134), 253 (1.745) nm. ^1H NMR (300.1 MHz, $CDCl_3$): δ = 8.34-8.24 (m, 2 H, Ar-*H*), 7.89-7.81 (m, 2 H, Ar-*H*), 7.31 (d, *J* = 1.9, 1 H, C*H*), 7.19 (d, *J* = 1.9, 1H, C*H*), 3.96 (s, 3 H, C*H*$_3$), 1.45 (s, 9 H, *t-Bu*) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 178.6 (N*C*O), 173.7 (N*C*N_{ox}), 171.3 (Au*C*N), 141.8 (N*C*$_{Ar}$), 129.5, 125.2, 125.2 (*C*H$_{Im}$), 122.8, 121.3 (*C*H$_{Im}$), 38.9 (*C*H_3), 32.5 (*C*CH_3), 28.4 (C*C*H_3) ppm. MS (EI): $C_{16}H_{18}AuClN_4O$ 514.1 (100, M^+), 479.1 (60), 423.1 (75), 380.1 (35), 282.1. Anal. Calcd for $C_{16}H_{18}AuClN_4O$: C, 37.33; H, 3.52; N, 10.88. Found: C, 37.52; H, 3.65; N, 10.67.

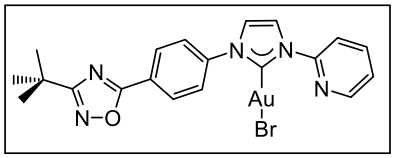
Bromo(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(benzyl)-1*H*-imidazolin-2(3*H*) ylidene) gold(I) (60)



Dry THF (5 mL) was added to a mixture of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(benzyl)-imidazolium bromide (88 mg, 0.2 mmol), potassium *tert*-butoxide (22.6 mg, 0.2 mmol) and (dimethyl sulfide)gold(I)chloride (59.5 mg, 0.2 mmol) under an inert atmosphere and the suspension was stirred for 24 h at room temperature in the dark. The resulting mixture was filtered over Celite. The solvent was removed in vacuo, and dichloromethane (2 mL) was added. The product was precipitated as a colorless solid upon addition of hexane and purified by column chromatography (DCM/ethyl acetate, 3:1). Yield*:* 78% (99 mg, 0.156 mmol). Single crystals suitable for X-ray diffraction were obtained by slow diffusion of pentane into a solution of **60** in dichloromethane.

IR (ATR): 1/λ = 3101, 2969, 2927, 2869, 1617, 1576, 1512, 1495, 1449, 1421, 1398, 1347, 1327, 1284, 1249, 1191, 1088, 1023, 989, 958, 900, 848, 775, 728, 695, 676, 633, 604, 581, 550 cm^{-1}. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 8.27-8.22 (m, 2 H, Ar-*H*), 7.92-7.86 (m, 2 H, Ar-*H*), 7.44-7.34 (m, 5 H, Ar-H_{Bn}), 7.29 (d, J_{HH} = 2.0 Hz, 1 H, CH_{Im}), 7.10 (d, J_{HH} = 2.0 Hz, 1 H, CH_{Im}), 5.46 (s, 2 H, C*H2*), 1.44 (s, 9 H, *t-Bu*) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 179.5 (N*C*O), 174.1 (N*C*N_{ox}), 173.7 (Au*C*N), 141.8 (NC_{Ar}), 134.3, 129.4, 129.1, 128.9, 128.3, 125.2, 125.1 (*C*H_{Im}), 121.7, 121.4 (*C*H_{Im}), 55.6 (*C*H_2-Bn), 32.5 (*C*CH_3), 28.4 (C*C*H_3) ppm.MS (EI): calcd. for $C_{22}H_{22}AuBrN_4O$: 634.0 / 636.0; found: M^+ 634.0 / 636.0; fragments: 555.0 (M-Br, 15), 357.1 (M-Br-Au, 100), 268.1 (M_{Im}, 35). MS (HR-ESI): calcd. for $C_{22}H_{22}AuBrN_4ONa$: 657.0534 (100), 658.0532 (20), 659.0516 (100), 660.0545 (20); found: 657.0523 (100), 658.0531 (20), 659.0502 (100), 660.0534 (20). Anal. Calcd for $C_{22}H_{22}AuBrN_4O$: C, 41.59; H, 3.49; N, 8.82. Found: C, 41.60; H, 3.59; N, 9.22.

Bromo(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2-pyridine)-1*H*-imidazolin-2(3*H*)-ylidene)gold(I) (61)

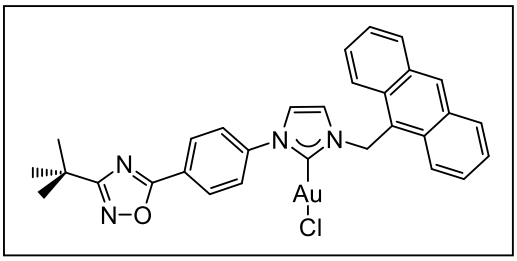


Dry THF (5 mL) was added to a mixture of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2-pyridine)-imidazolium bromide (86 mg, 0.2 mmol), potassium *tert*-butoxide (22.6 mg, 0.2 mmol) and (dimethyl sulfide)chlorogold(I) (59.5 mg, 0.2 mmol) under an inert atmosphere and the suspension was stirred for 24 h at room temperature in the dark. The resulting mixture was filtered over Celite. The solvent was removed in vacuo and dichloromethane (2 mL) was added. The product was precipitated as a

colorless solid upon addition of hexane and purified by column chromatography (DCM/ethyl acetate, 3:1). Yield*:* 85% (105.8 mg, 0.17 mmol). Single crystals suitable for X-ray diffraction were obtained by slow evaporation of a concentrated solution of **61** in dichloromethane.

IR (ATR): 1/λ = 3172, 3130, 3105, 2962, 2930, 2869, 1612, 1590, 1512, 1469, 1440, 1421, 1390, 1349, 1316, 1280, 1196, 1177, 1091, 1046, 994, 949, 888, 852, 777, 740, 716, 698, 681, 646, 609, 580, 538 cm^{-1}. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 8.83 (m, 1 H, CH_{Py}), 8.57 (m, 1 H, CH_{Py}), 8.33-8.28 (m, 2 H, Ar-*H*), 8.03 (d, J_{HH} = 2.0 Hz, 1 H, CH_{Im}), 7.99-7.92 (m, 3 H, CH_{Py}, Ar-*H*), 7.48 (d, J_{HH} = 2.0 Hz, 1 H, CH_{Im}), 7.47-7,44 (m, 1 H, CH_{Py}), 1.44 (s, 9 H, *t-Bu*) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 178.5 (N*C*O), 173.6 (N*C*N_{ox}), 172.7 (Au*C*N), 150.1 (C_{Py}), 148.9 (*C*H_{Py}), 141.9 (*C*H_{Py}), 139.1 (NC_{Ar}), 129.5 (CH_{Ar}), 125.5 (C_{Ar}), 125.5 (*C*H_{Py}), 124.4 (*C*H_{Ar}), 121.7 (*C*H_{Im}), 121.4 (*C*H_{Im}), 117.3 (*C*H_{Py}), 32.5 (*C*CH_3), 28.4 (C*C*H_3) ppm. MS (HR-EI): calcd. for $C_{20}H_{19}AuBrN_5O$: 621.0438; found: M^+ 621.0433; fragments: 542.0 (M-Br, 35), 345.1 (M-Br-Au, 100), 288.1 (60). Anal. Calcd for $C_{20}H_{19}AuBrN_5O$: C, 38.60; H, 3.08; N, 11.25. Found: C, 38.65; H, 3.23; N, 11.31.

Chloro(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(9-methyl-anthracene)-1*H*-imidazolin -2(3*H*) -ylidene)gold(I) (62)

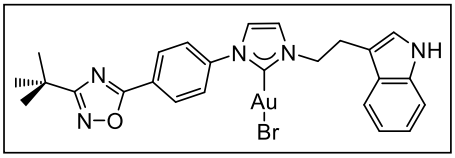


Dry THF (5 mL) was added to a mixture of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(9-methyl-anthracene)-imidazolium chloride (100 mg, 0.2 mmol), potassium *tert*-butoxide (22.6 mg, 0.2 mmol) and (dimethyl sulfide)chlorogold(I) (59.5 mg, 0.2 mmol) under an inert atmosphere and the suspension was stirred for 24 h at room temperature in the dark. The resulting mixture was filtered over Celite. The solvent was removed in vacuo, and dichloromethane (2 mL) was added. The product was precipitated as a colorless solid upon addition of hexane and purified by column chromatography (DCM/ethyl acetate, 3:1). Yield*:* 63% (87 mg, 0.126 mmol). Single crystals suitable for X-ray diffraction were obtained by slow evaporation of a concentrated solution of **62** in dichloromethane.

IR (ATR): 1/λ = 3130, 3053, 2968, 2928, 2870, 1616, 1594, 1572, 1511, 1495, 1464, 1425, 1396, 1348, 1324, 1283, 1229, 1196, 1117, 1089, 1019, 957, 902, 850, 798, 775, 727, 700, 678, 649, 630, 601, 574, 553. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 8.58 (s, 1 H, CH_{An}), 8.33-8.27 (m, 2 H, CH_{An}), 8.22-8.17 (m, 2 H, Ar-*H*), 8.12-8.06 (m, 2 H, CH_{An}), 7.94-7.86 (m, 2 H, Ar-*H*), 7.67-7.51 (m, 4 H, CH_{An}), 7.01 (d, J_{HH} = 2 Hz, 1 H, CH_{Im}), 6.49 (d, J_{HH} = 2 Hz, 1 H,

C*H*$_{Im}$), 6.32 (s, 2 H, C*H*$_2$), 1.43 (s, 9 H, *t-Bu*).^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 178.4 (N*C*O), 173.6 (N*C*N$_{ox}$), 170.6 (Au*C*N), 142.1 (N*C*$_{Ar}$), 131.3, 130.9, 130.2, 129.5, 129.4, 127.9, 125.5, 125.3, 125.1 (*C*H$_{Im}$), 123.2, 123.0, 121.1, 120.8 (*C*H$_{Im}$), 47.6 (*C*H$_2$-An), 32.5 (*C*CH$_3$), 28.4 (C*C*H$_3$). MS (HR-EI): calcd. for $C_{30}H_{26}AuClN_4O$: 690.1461; found: 690.1455. Anal. Calcd for $C_{30}H_{26}AuClN_4O$: C, 52.15; H, 3.79; N, 8.11. Found: C, 51.94; H, 3.83; N, 8.14.

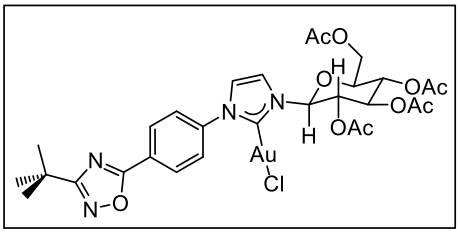
Bromo(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-((2-ethyl)indole)-1*H*-imidazolin-2(3*H*)-ylidene)gold(I) (63)



Dry THF (5 mL) was added to a mixture of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-((2-ethyl)indole)-imidazolium bromide (98.5 mg, 0.2 mmol), potassium *tert*-butoxide (22.6 mg, 0.2 mmol) and (dimethyl sulfide)chlorogold(I) (59.5 mg, 0.2 mmol) under an inert atmosphere and the suspension was stirred for 24 h at room temperature in the dark. The resulting mixture was filtered over Celite. The solvent was removed in vacuo, and dichloromethane (2 mL) was added. The product was precipitated as a colorless solid upon addition of hexane and purified by column chromatography (DCM/ethyl acetate, 3:1). Yield*:* 46% (64 mg, 0.092 mmol).

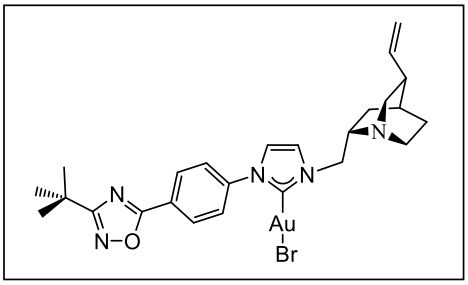
IR (ATR): 1/λ = 3350, 3127, 3058, 2969, 2927, 2868, 1616, 1512, 1457, 1422, 1399, 1350, 1279, 1227, 1195, 1093, 1013, 959, 849, 775, 739, 699, 678, 636, 602, 585, 555. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 8.31 (s, 1 H, N*H*), 8.21-8.14 (m, 2 H, Ar-*H*), 7.67-7.61 (m, 2 H, Ar-*H*), 7.47-7.35 (m, 2 H, C*H*$_{In}$), 7.22 (m, 1 H, C*H*$_{In}$), 7.09-7.01 (m, 3 H, C*H*$_{Im}$, C*H*$_{In}$, C*H*$_{In}$), 6.84 (d, *J* = 2.0, 1H, C*H*$_{Im}$), 4.50 (t, 2 H, C*H*$_2$), 3.34 (t, 2 H, C*H*$_2$), 1.44 (s, 9 H, *t-Bu*). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 178.5 (N*C*O), 173.8 (N*C*N$_{ox}$), 173.7 (Au*C*N), 141.9 (N*C*$_{Ar}$), 136.1, 129.3, 127.2, 125.2, 125.1, 124.9, 122.9, 122.2, 122.0, 120.6, 119.6, 117.9, 111.5 (Ar-*C*H$_{In}$), 110.8 (CH$_2$-*C*$_{In}$), 52.9 (*C*H$_2$), 32.5 (*C*CH$_3$), 28.4 (C*C*H$_3$), 27.2 (*C*H$_2$). MS (EI): calcd. for $C_{25}H_{25}AuBrN_5O$ 687.0 / 689.0; found: M^+ 687.0 / 689.0 ;fragments: 608.1 (M-Br, 10), 411.1 (M-Br-Au, 15), 268.1 (M$_{Im}$, 55). Anal. Calcd for $C_{25}H_{25}AuBrN_5O.C_5H_{12}$: C, 47.38; H, 4.90; N, 9.21. Found: C, 47.06; H, 4.47; N, 9.84.

Chloro(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2,3,4,5-tetra-*O*-acetyl-*D*-glucopyranosyl)-1*H*-imidazolin-2(3*H*)-ylidene)gold(I) (64)



Dry THF (5 mL) was added to a mixture of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(2,3,4,5-tetra-*O*-acetyl-*D*-glu-copyranosyl)-imidazolium nitrate (132 mg, 0.2 mmol), potassium *tert*-butoxide (22.6 mg, 0.2 mmol) and (dimethyl sulfide)gold(I)chloride (59.5 mg, 0.2 mmol) under an inert atmosphere and the suspension was stirred for 24 h at room temperature in the dark. The resulting mixture was filtered over Celite. The solvent was removed in vacuo, and dichloromethane (2 mL) was added. The product was precipitated as a colorless solid upon addition of hexane and purified by column chromatography (DCM/ethyl acetate, 3:1). Yield*:* 52% (85 mg, 0.104 mmol).
IR (ATR): 1/λ = 3095, 2963, 2928, 2354, 2332, 1748, 1745, 1615, 1513, 1424, 1391, 1362, 1342, 1328, 1311, 1239, 1212, 1187, 1154, 1110, 1081, 1065, 1048, 1011, 952, 935, 911, 885, 842, 822, 767, 733, 691, 651, 615 cm^{-1}. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 8.36-8.28 (m, 2 H, Ar-*H*), 7.86-7.79 (m, 2 H, Ar-*H*), 7.50 (d, J_{HH} = 2.1 Hz, 1 H, CH_{Im}), 7.36 (d, J_{HH} = 2.1 Hz, 1 H, CH_{Im}), 6.17 (d, J_{HH} = 8.5 Hz, 1 H, N-*CH*-O), 5.58 (d, J_{HH} = 3.1 Hz, 1 H, CH_2), 5.45-5.31 (m, 2 H, 2 x $H_{carbohydrate}$), 4.39-4.28 (m, 1 H, C$H_{carbohydrate}$), 4.27-4.15 (m, 2 H, C$H_{carbohydrate}$, CH_2), 2.23 (s, 3 H, CH_3), 2.09 (s, 3 H, CH_3), 2.07 (s, 3 H, CH_3), 2.03 (s, 3 H, CH_3), 1.45 (s, 9 H, *t-Bu*) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 178.6 (N*C*O), 173.5 (N*C*N_{ox}), 170.9 (Au*C*N), 170.3 (CH_3-*C*=O), 169.8 (CH_3-*C*=O), 169.7 (CH_3-*C*=O), 169.4 (CH_3-*C*=O), 141.5 (C_{Ar}), 129.6 (*C*H_{Ar}), 125,7 (*C*H_{Im}), 125.2 (*C*H_{Ar}), 122.4 (C_{Ar}), 118.9 (*C*H_{Im}), 87.4 (N-*C*$H_{carbohydrate}$-O), 74.0 (O-*C*$H_{carbohydrate}$-CH_2), 70.2 (*C*$H_{carbohydrate}$), 68.6 (*C*$H_{carbohydrate}$), 66.8 (*C*$H_{carbohydrate}$), 61.3 (CH-*C*$H_{2carbohydrate}$-OAc), 32.5 (*C*-CH_3), 28.4 (C-*C*H_3), 20.8 (COO-*C*H_3), 20.6 (COO-*C*H_3), 20.4 (COO-*C*H_3) ppm. MS (HR-ESI): calcd. for $C_{29}H_{34}AuClN_4O_{10}Na$: 853.1521 (100), 854.1552 (30), 855.1508 (30), 856.1531 (10); found: 853.1530 (100), 854.1558 (30), 855.1510 (40), 856.1530 (10). MS (EI): calcd. for $C_{29}H_{34}AuClN_4O_{10}$ 830.2 /832.2; found: M^+ 830.2 / 832.2; fragments: 795.2 (M-Cl, 70), 595.1 (45), 553.1 (100), 493.1 (80). Anal. Calcd for $C_{29}H_{34}AuClN_4O_{10}$: C, 41.91; H, 4.12; N, 6.74. Found: C, 42.19; H, 4.22; N, 6.86.

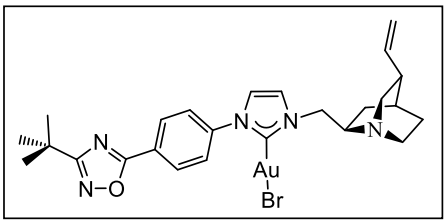
Bromo(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCI)-1*H*-imidazolin-2(3*H*)-ylidene)gold(I) (65)



Dry THF (5 mL) was added to a mixture of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCI)-imidazolium bromide (100 mg, 0.2 mmol), potassium *tert*-butoxide (22.6 mg, 0.2 mmol) and (dimethyl sulfide)gold(I)chloride (59.5 mg, 0.2 mmol) under an inert atmosphere and the suspension was stirred for 24 h at room temperature in the dark. The resulting mixture was filtered over Celite. The solvent was removed in vacuo and dichloromethane (2 mL) was added. The product was precipitated as a colorless solid upon addition of hexane and purified by column chromatography (DCM/MeOH, 15:1). Yield*:* 41% (57 mg, 0.082 mmol). Single crystals suitable for X-ray diffraction were obtained by slow diffusion of pentane into a solution of **65** in dichloromethane.

IR (ATR): 1/λ = 3171, 3123, 3095, 2966, 2920, 2867, 2851, 1958, 1929, 1825, 1730, 1688, 1665, 1635, 1614, 1591, 1574, 1560, 1524, 1512, 1496, 1464, 1450, 1424, 1407, 1392, 1367, 1349, 1327, 1290, 1272, 1259, 1244, 1195, 1177, 1137, 1124, 1100, 1065, 1051, 1037, 989, 956, 941, 909, 892, 871, 853, 821, 793, 776, 745., 726., 699, 676., 663, 636, 618 cm^{-1}. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 8.29-8.23 (m, 2 H, Ar-*H*), 7.91-7.86 (m, 2 H, Ar-*H*), 7.51 (d, J_{HH} = 1.98 Hz, 1 H, C*H*$_{Im}$), 7.28 (d, J_{HH} = 1.98 Hz, 1 H, C*H*$_{Im}$), 5.82 (ddd, J_{HH} = 17.40, J_{HH} = 10.32, J_{HH} = 7.4, 1 H, H-10), 5.06-4.99 (m, 2 H, H-11, H-11), 4.17 (d, J = 7.05 Hz, 2 H, H-9), 3.53-3.36 (m, 2 H; H-2, H-6), 3.31-3.16 (m, 1 H; H-7), 2.89-2.81 (m, 1 H, H-6), 2.72-2.59 (m, 1 H, H-7), 2.49-1.35 (m, 1 H, H-5), 2.18-2.03 (m, 1 H, H-3), 1.92-1.85 (m, 1 H, H-4), 1.77-1.68 (m, 2 H, H-8, H-8), 1.44 (s, 9 H, *t-Bu*), 0.90-0.83 (m, 1 H, H-3) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 181.8 (Au*C*N), 178.5 (N*C*O), 173.7 (N*C*N$_{ox}$), 141.9 (*C*H, C-10), 140.1 (N*C*$_{Ar}$), 129.4 (*C*H$_{Ar}$), 125.3, 125.1 (*C*H$_{Im}$), 122.2, 121.5 (*C*H$_{Im}$), 115.6 (*C*H$_2$, C-11), 57.94 (*C*H$_2$, C-9), 56.9 (*C*H, C-2), 52.9 (*C*H$_2$, C-7), 42.2 (*C*H$_2$, C-6), 38.7 (*C*H, C-4), 32.5 (*C*CH$_3$), 28.4 (C*C*H$_3$), 27.0 (*C*H$_2$, C-8), 26.9 (*C*H, C-5), 25.8 (*C*H$_2$, C-3) ppm. MS (HR-ESI): calculated for $C_{25}H_{31}AuN_5O + H^+$: 694.1450 (100), 695.1484 (30), 696.1430 (100), 697.1463 (30), 698.1496 (5); found: 694.1439 (100), 695.1470 (30), 696.1419 (100), 697.1446 (30), 698.1479 (5). MS (EI): 693.1 / 695.1 (M^+, 100), 614.2 (M^+ - Br), 560.1 (M^+- Br - *t*Bu), 417.2 (M^+ - AuBr). Anal. Calcd for $C_{25}H_{25}AuBrN_5O.CH_2Cl_2$: C, 40.07; H, 4.27; N, 8.99. Found: C, 39.73; H, 4.43; N, 8.66.

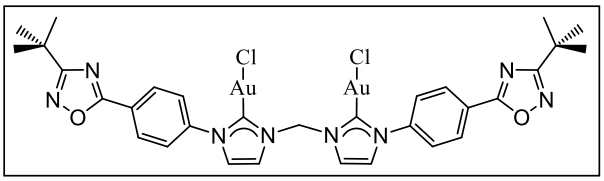
Bromo(1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCD)-1*H*-imidazolin-2(3*H*)-ylidene)gold(I) (66)



Dry THF (5 mL) was added to a mixture of 1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-(QCD)-imidazolium bromide (100 mg, 0.2 mmol), potassium *tert*-butoxide (22.6 mg, 0.2 mmol) and (dimethyl sulfide)gold(I)chloride (59.5 mg, 0.2 mmol) under an inert atmosphere and the suspension was stirred for 24 h at room temperature in the dark. The resulting mixture was filtered over Celite. The solvent was removed in vacuo and dichloromethane (2 mL) was added. The product was precipitated as a colorless solid upon addition of hexane and purified by column chromatography (DCM/MeOH, 15:1). Yield*:* 47% (65 mg, 0.093 mmol).

IR (ATR): 1/λ = 3116, 3095, 2972, 2942, 2903, 2870, 2857, 1953, 1906, 1725, 1694, 1637, 1614, 1591, 1572, 1560, 1512, 1496, 1465, 1452, 1424, 1394, 1348, 1324, 1308, 1242, 1229, 1195, 1124, 1088, 1059, 1029, 986, 958, 914, 891, 849, 798, 776, 754, 734, 722, 698, 678, 636, 621 cm^{-1}. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 8.29-8.24 (m, 2 H, Ar-*H*), 7.92-7.86 (m, 2 H, Ar-*H*), 7.45 (d, J_{HH} = 1.8 Hz, 1 H, CH_{Im}), 7.26 (d, J_{HH} = 1.9 Hz, 1 H, CH_{Im}), 5.98-5.84 (ddd, J_{HH} = 16.8 Hz, J_{HH} = 11.05 Hz, J_{HH} = 9.15 Hz, 1 H, *H-10*), 5.19-5.11 (m, 2 H, *H*-11, *H*-11), 4.45 (dd, J_{HH} = 14.1 Hz, J_{HH} = 4.7 Hz, 1 H, *H*-9), 4.37-4.21 (m, 1 H, *H*-9), 3.49-3.33 (m, 1 H; *H*-2), 3.19-2.78 (m, 4 H; *H*-6, *H*-6, *H*-7, *H*-7), 2.48-2.33 (m, 1 H; *H*-5), 1.89-1.83 (m, 1 H; *H*-4), 1.79-1.62 (m, 3 H, *H*-3, *H*-8, *H*-8), 1.44 (s, 9 H, *t-Bu*), 1.27-1.23 (m, 1 H, *H*-3) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 181.9 (Au*C*N), 178.5 (N*C*O), 173.7 (N*C*N_{ox}), 141.9 (*C*H, C-10), 139.0 (NC_{Ar}), 129.4 (*C*H_{Ar}), 125.2, 125.1 (*C*H_{Im}), 122.6, 121.1 (*C*H_{Im}), 115.5 (*C*H_2, C-11), 57.7 (*C*H_2, C-9), 56.6 (*C*H, C-2), 52.4 (*C*H_2, C-7), 49.1 (*C*H_2, C-6), 39.1 (*C*H, C-4), 32.5 (*C*CH_3), 28.4 (C*C*H_3), 27.1 (*C*H_2, C-8), 25.9 (*C*H, C-5), 25.2 (*C*H_2, C-3) ppm. MS (HR-ESI): calculated for $C_{25}H_{31}AuN_5O + H^+$: 694.1450 (100), 695.1484 (30), 696.1430 (100), 697.1463 (30), 698.1496 (5); found: 694.1429 (100), 695.1459 (30), 696.1409 (100), 697.1436 (30), 698.1467 (5). MS (EI): 693.1 / 695.1 (M^+, 100), 614.2 (M^+ - Br), 560.1 (M^+- Br - *t*Bu), 417.2 (M^+ - AuBr). Anal. Calcd for $C_{25}H_{25}AuBrN_5O.CHCl_3$: C, 38.37; H, 3.96; N, 8.61. Found: C, 38.90; H, 4.29; N, 8.29.

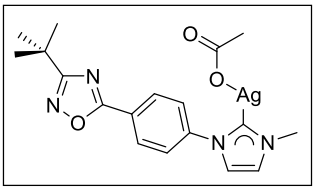
Dichloro{1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylene-diimidazolin-2,2′-diylidene}digold(I) (67)



A mixture of 1,1′-[4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl]-3,3′-methylene di-imidazolium bis(bromide) (100 mg, 0.140 mmol) and silver(I) oxide (39.16 mg, 0.169 mmol) in dry dichloromethane (20 mL) was stirred for 36 h at room temperature in the dark. The mixture was filtered over Celite, and AuCl(THT) (41.23 mg, 0.140 mmol) was added. The resulting mixture was stirred for 12 h and filtered over Celite. The solvent was removed in vacuo, and dichloromethane (2 mL) was added. The product was precipitated as a colorless solid upon addition of pentane and purified by column chromatography (DCM/ethyl acetate, 3:1). Yield*:* 35% (49.5 mg, 0.049 mmol).

IR (ATR): 1/λ = 3496, 3305, 3102, 2967, 2926, 2869, 1691, 1614, 1512, 1462, 1427, 1396, 1349, 1279, 1252, 1232, 1194, 1093, 1063, 1020, 991, 958, 892, 850, 775, 723, 698, 677, 634, 603, 557 cm^{-1}. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 8.34-8.27 (m, 4 H, Ar-*H*), 8.15 (d, *J* = 1.95, 2 H, C*H*), 7.91-7.83 (m, 4 H, Ar-*H*), 7.35 (d, *J* = 1.95, 2 H, C*H*), 6.62 (s, 2 H, C*H2*), 1.44 (s, 18 H, *t-Bu*) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 178.0 (N*C*O), 173.1 (N*C*N_{ox}), 171.9 (Au*C*N_{Im}), 141.1 (N*C*$_{Ar}$), 129.8 (*C*H$_{Ar}$), 128.2 (*C*$_{Ar}$), 125.7 (CH*C*HN_{Im}), 124.9 (*CH*$_{Ar}$), 121.9 (*C*HCHN_{Im}), 63.2 (N*C*H_2N), 32.2 (*C*CH_3), 28.1 (C*C*H_3) ppm. MS (ESI): $C_{31}H_{32}Au_2Cl_2N_8O_2$ + Na^+ 1035.13 (100), 1037.00 (70), 977.33 (M-Cl, 60); (HR-ESI): calcd. for $C_{31}H_{32}Au_2ClN_8O_2$ (M-Cl) 977.1668 (100), 978.1701, (35), 979.1638, (30), 980.1672 (10); found 977.1667 (100), 978.1700, (35), 979.1639, (30), 980.1672 (10). Anal. Calcd for $C_{31}H_{32}Au_2Cl_2N_8O_2$.0.5 AuCl(THT): C 33.77; H, 3.09; N, 9.55. Found: C, 33.72; H, 3.14; N, 9.94.

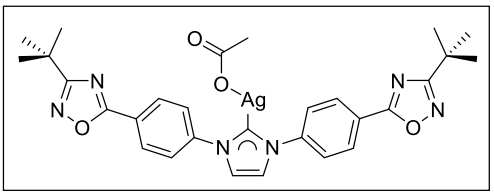
Synthesis of (1-(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-3-methyl-1,3-dihydro-2H-imidazol-2-ylidene)argentio acetate (68)



1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-methyl-imidazolium iodide (0.198 mmol, 81.23 mg) and silver(I) acetate (0.495 mmol, 82.67 mg) were mixed in a round-bottomed flask containing 3 Å molecular sieves and dry CH_2Cl_2 (20 mL). The reaction mixture was stirred at ambient temperature for 48 h in the absence of light. The suspension was then filtered through Celite, and the solvent was removed under reduced pressure to afford the desired product as a yellow-grey solid. Yield*:* 94% (83.6 mg, 0.186 mmol).

IR (ATR): 1/λ = 3370, 3164, 3080, 2970, 2873, 1613, 1568, 1511, 1466, 1421, 1395, 1349, 1269, 1243, 1196, 1105, 1092, 1012, 955, 922, 889, 849, 775, 743, 697, 668, 616, 603, 548 cm^{-1}. UV/Vis (DCM): λmax. (lg ε) = 193 (0.802), 196 (0.700), 266 (0.500), 358 (0.011) nm. ^{1}H NMR (300.1 MHz, $CDCl_3$): 8.22-8.14 (m, 2 H, Ar-*H*), 7.72-7.65 (m, 2 H, Ar-*H*), 7.29 (d, *J* = 1.8, 1H, C*H*$_{imidazol}$), 7.13 (d, *J* = 1.8, 1H, C*H*$_{imidazol}$), 3.89 (s, 3H, C*H*$_3$), 1.96 (s, 3H, COC*H*$_3$), 1.35 (s, 9 H, *t-Bu*) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 179.85 (Ag*C*N), 178.52 (CH_3*C*OO), 178.12 (N*C*O), 173.71 (N*C*N), 142.61 (N*C*N), 129.71 (*C*H$_{Ar}$), 125.01 (*C*$_{Ar}$), 124.25 (*C*H$_{Ar}$), 123.27 (*C*$_{Ar}$), 121.45 (*C*H$_{im}$), 39.39 (*C*(CH_3)$_3$), 32.53 (NC*H*$_3$), 28.41 (C(*C*H$_3$)$_3$), 22.81 (COO*C*H_3) ppm. MS (ESI): 283.2 (M-AgCOOCH_3 + H^+, 100). $C_{18}H_{21}AgN_4O_3$ (448.07): calcd. C 48.12, H 4.71, N 12.47; found C 47.25, H 4.84, N 11.60.

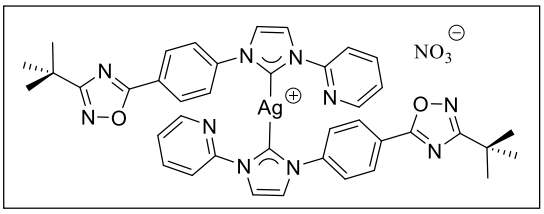
Synthesis of 1-((1,3-bis(4-(3-(*tert*-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-1*H*-imidazol-2(3*H*)-ylidene)argentio acetate (69)



1,3-Bis(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole) imidazolium chloride (0.198 mmol, 100 mg) and silver(I) acetate (0.495 mmol, 82.67 mg) were mixed in a round-bottomed flask containing 3 Å molecular sieves and dry CH_2Cl_2 (20 mL). The reaction mixture was stirred at ambient temperature for 48 h in the absence of light. The suspension was then filtered through Celite, and the solvent was removed under reduced pressure to afford the desired product as a yellow-grey solid. Yield*:* 87% (109.4 mg, 0.172 mmol).

IR (ATR): 1/λ = 3361, 3121, 2968, 2929, 2871, 1613, 1568, 1511, 1465, 1415, 1395, 1350, 1280, 1260, 1195, 1083, 1016, 944, 891, 849, 800, 774, 736, 698, 671, 609, 572 cm^{-1}. ^{1}H-NMR (300.1 MHz, $CDCl_3$): 8.39-8.31 (m, 4 H, Ar-*H*), 7.94-7.85 (m, 4 H, Ar-*H*), 7.60 (s, 2 H, C*H*$_{imidazol}$), 2.01 (s, 3 H, C*H3*), 1.45 (s, 18 H, *t-Bu*) ppm. ^{13}C-NMR (75.47 MHz, $CDCl_3$): δ = 179.04 (Ag*C*N), 178.60 (CH_3*C*OO, N*C*O), 173.63 (N*C*N), 142.39 (*C*$_{Ar}$), 129.94 (*C*H$_{Ar}$), 125.60 (*C*H$_{Ar}$), 124.46 (*C*$_{Ar}$), 122.53 (*C*H$_{imidazol}$), 32.57 (*C*-(CH_3)$_3$), 28.43 (C(*C*H$_3$)$_3$), 22.60 (COO*C*H_3) ppm. MS (ESI): 469.2 (M-AgCOOCH_3 + H^+, 100), 413.2 (M-AgCOOCH_3 - *t*-Bu + H^+, 60), 357.2 [M-AgCOOCH_3 – 2 x *t*-Bu +H^+, 55].

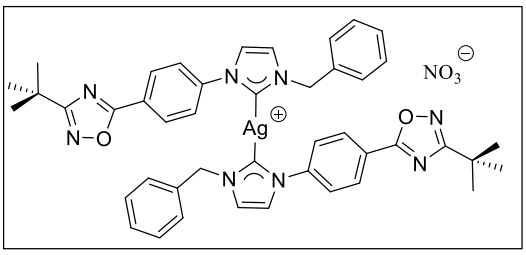
Synthesis of bis(1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(2-pyridine)-imidazolium-2-ylidene) silver (I) nitrate (70)



A mixture of 1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(2-pyridine)-imidazolium bromide (100 mg, 0.23 mmol) and $AgNO_3$ (0.040 g, 0.23 mmol) were stirred in CH_2Cl_2 (10 mL) for 30 minutes at room temperature. The mixture was filtered and the volatile components were removed under vacuum. The resulting solid was washed with Et_2O. The Et_2O was decanted and the product was dried under vacuum. Yield*:* 87% (81.6 mg, 0.2 mmol). Ag_2O (46 mg, 0.2 mmol) was added to a solution of 1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(2-pyridine)-imidazolium Nitrate (0.80 g, 0.2 mmol) in DCM (30 mL) and the mixture was stirred at room temperature for 24 h. After filtration through Celite a clear solution was obtained. The volatile components were removed under vacuum and the resulting solid was washed with OEt_2 and dried to yield a white-grey solid. Yield*:* 47% (41 mg, 0.047 mmol).

^{1}H NMR (300.1 MHz, DMSO-d_6): δ = 8.48 (m, 2H, CH_{Py}), 8.29 (m, 2H, CH_{Py}), 8.21 (m, 2H, CH_{Py}), 8.15-7.95 (m, 12H, Ar-*H*, CH_{Im}), 7.51-7,44 (m, 2H, CH_{Py}), 1.42 (s, 18H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 178.43 (Ag*C*N), 173.46 (N*C*N$_{ox}$), 172.81 (N*C*O), 155.26 (*C*$_{Py}$), 148.58 (*C*H$_{Py}$), 143.39 (*C*H$_{Py}$), 141.09 (N*C*$_{Ar}$), 128.45 (*C*H$_{Ar}$), 124.50 (*C*$_{Ar}$), 124.25 (*C*H$_{Py}$), 123.41 (*C*H$_{Ar}$), 121.64 (*C*H$_{Im}$), 121.16 (*C*H$_{Im}$), 115.75 (*C*H$_{Py}$), 32.14 (*C*CH$_3$), 28.10 (C*C*H$_3$) ppm. MS (HR-ESI): calcd. for $C_{40}H_{38}AgN_{10}O_2^+$: 797.2224 (100), 798.2253 (45), 799.2227 (95), 800.2252 (45), 801.2279 (10); found: 797.2231 (100), 798.2268 (45), 799.2226 (95), 800.2262 (45), 801.2304 (10).

Synthesis of bis(1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(benzyl)-imidazolium -2-ylidene) silver (I) nitrate (71)

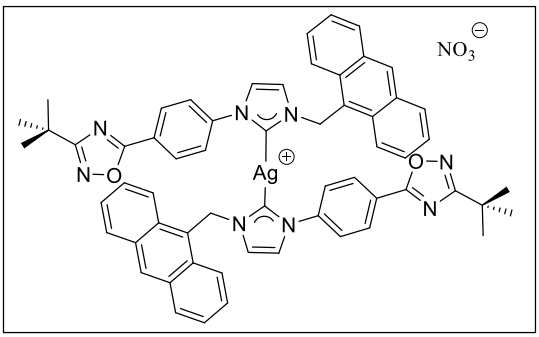


A mixture of 1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(benzyl)-imidazolium bromide (176 mg, 0.4 mmol) and $AgNO_3$ (0.078 g, 0.4 mmol) were stirred in CH_2Cl_2 (10 mL) for 30 minutes at room temperature. The mixture was filtered and the volatile components were removed under vacuum. The resulting solid was washed with OEt_2. The OEt_2 was decanted and the product was dried under vacuum. Yield*:* 93% (157 mg, 0.372 mmol). Ag_2O (85 mg, 0.36 mmol) was added to a solution of 1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(benzyl)-imidazolium Nitrate (0.15 g, 0.36 mmol) in CH_3CN (30 mL) and

the mixture was stirred at room temperature for 24 h. After filtration through Celite a clear solution was obtained. The volatile components were removed under vacuum and the resulting solid was washed with OEt_2 and dried to yield a light-yellow solid. Yield*:* 58% (96 mg, 0.108 mmol).

IR (ATR): 1/λ = 3469, 3126, 3095, 2969, 2929, 2871, 1615, 1512, 1496, 1454, 1417, 1394, 1349, 1320, 1283, 1246, 1195, 1103, 1085, 1029, 956, 893, 851, 775, 739, 713, 637, 603, 578, 550 cm^{-1}. ^1H NMR (300.1 MHz, $CDCl_3$): δ = 8.19-8.13 (m, 4H, Ar-*H*), 7.77-7.71 (m, 4H, Ar-*H*), 7.43 (d, J_{HH} = 1.9 Hz, 2H, CH_{Im}), 7.34-7.26 (m, 12H, 7.30 d, J_{HH} = 1.9 Hz CH_{Im}, Ar-H_{Bn}), 5.36 (s, 4H, CH_2), 1.44 (s, 18H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 179.03 (Ag*C*N), 178.43 (N*C*O), 173.55 (N*C*N_{Ox}), 142.63 (NC_{Ar}), 135.04, 129.58, 129.14, 128.76, 127.89, 124.95 (CH_{Im}), 124.32, 122.94, 121.99 (*C*H_{Im}), 56.16 (*C*H_2-Bn), 32.48 (*C*CH_3), 28.38 (C*C*H_3) ppm. MS (HR-ESI): calcd. for $C_{44}H_{44}AgN_8O_2^+$: 823.2632 (100), 824.2672 (50), 825.2637 (95), 826.2662 (50), 827.2690 (10); found: 823.2613 (100), 824.2663 (50), 825.2608 (95), 826.2629 (50), 827.2658 (10).

Synthesis of bis(1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(9-methyl-antracene)-imidazolium -2-ylidene) silver (I) nitrate (72)

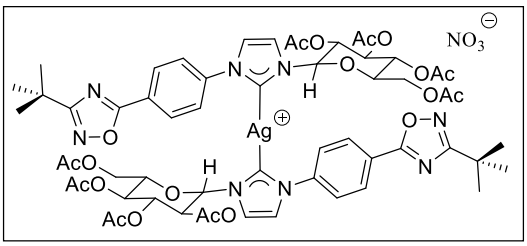


A mixture of 1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(9-methyl-antracene)-imidazolium chloride (200 mg, 0.4 mmol) and $AgNO_3$ (0.078 g, 0.4 mmol) were stirred in CH_2Cl_2 (10 mL) for 30 minutes at room temperature. The mixture was filtered and the volatile components were removed under vacuum. The resulting solid was washed with Et_2O. The Et_2O was decanted and the product was dried under vacuum. Yield*:* 96% (200 mg, 0.384 mmol). Ag_2O (88 mg, 0.38 mmol) was added to a solution of 1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(9-methyl-antracene)-imidazolium Nitrate (0.2 g, 0.38 mmol) in CH_3CN (30 mL) and the mixture was stirred at room temperature for 24 h. After filtration through Celite a clear solution was obtained. The volatile components were removed under vacuum and the resulting solid was washed with Et_2O and dried to yield a light-yellow solid. Yield*:* 56% (116 mg, 0.106 mmol).

IR (ATR): 1/λ = 3461, 3057, 2969, 2928, 2870, 1615, 1512, 1418, 1392, 1349, 1281, 1232, 1195, 1105, 1027, 954, 892, 848, 796, 775, 727, 700, 628, 601, 551 cm^{-1}. ^1H NMR (300.1

MHz, CD_3CN): δ = 8.42 (s, 2H, CH_{An}), 8.22-8.15 (m, 4H, CH_{An}), 7.96-7.85 (m, 8H, CH_{An}, Ar-*H*), 7.54-7.32 (m, 16H, Ar-*H*, CH_{An}, CH_{Im}), 6.05 (s, 4H, CH_2), 1.41 (s, 18H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, CD_3CN): δ = 180.59 (Ag*C*N), 179.31 (N*C*O), 174.82 (N*C*N_{ox}), 143.89 (N*C*$_{Ar}$), 132.18, 131.58, 130.69, 130.35, 129.88, 128.43, 126.19, 126.05, 125.23, 125.18, 124.32, 124.24 (CH_{Im}), 122.16 (CH_{Im}), 48.39 (*C*H_2-An), 33.21 (*C*CH_3), 28.71 (C*C*H_3) ppm. MS (HR-ESI): calcd. for $C_{60}H_{52}AgN_8O$: 1023.3264 (98), 1024.3298 (60), 1025.3261 (100), 1026.3289 (60), 1027.3320 (20), 1028.3351 (10); found: 1023.3272 (90), 1024.3293 (60), 1025.3275 (100), 1026.3295 (60), 1027.3324 (20), 1028.3354 (10).

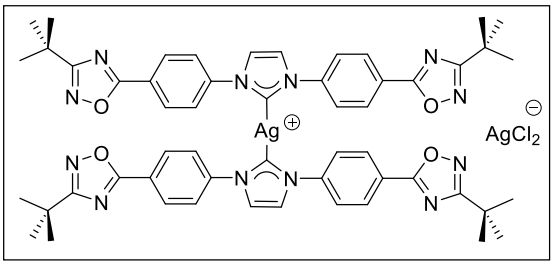
Synthesis of bis(1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(2,3,4,5-tetra-*O*-acetyl-*D*-glucopyranosyl)-imidazolium-2-ylidene) silver (I) nitrate (73)



A mixture of 1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(2,3,4,5-tetra-*O*-acetyl-*D*-gluco-pyranosyl)-imidazolium nitrate (265 mg, 0.4 mmol) and Ag_2O (89 mg, 0.4 mmol) in CH_3CN (30 mL) was stirred at room temperature for 48 h. After filtration through Celite a clear solution was obtained. The volatile components were removed under vacuum and the resulting solid was washed with OEt_2 and dried to yield a white-yellow solid. Yield*:* 67% (366 mg, 0.268 mmol).

IR (ATR): 1/λ = 3134, 2972, 1747, 1616, 1513, 1420, 1367, 1210, 1087, 1056, 953, 919, 852, 776, 739, 700, 652, 597, 554, 534 cm^{-1}. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 8.31-8.24 (m, 4H, Ar-*H*), 7.82-7.75 (m, 4H, Ar-*H*), 7.59 (d, J_{HH} = 1.9 Hz, 2H, CH_{Im}), 7.51 (d, J_{HH} = 1.9 Hz, 2H, CH_{Im}), 6.12 (d, J_{HH} = 8.1 Hz, 2H, NC*H*O), 5.60 (d, J_{HH} = 2.0 Hz, 2H, CH_2), 5.52-5.41 (m, 4H, 4 x C$H_{carbohidrate}$), 4.57-4.47 (m, 2H, C$H_{carbohidrate}$), 4.32-4.16 (m, 4H, C$H_{carbohydrate}$, CH_2), 2.23 (s, 6H, CH_3), 2.05 (s, 6H, CH_3), 2.01 (s, 6H, CH_3), 1.98 (s, 6H, CH_3), 1.44 (s, 18H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 179.01 (Ag*C*N),178.33 (N*C*O), 173.42 (N*C*N_{ox}), 170.25 (CH_3CO), 169.78 (CH_3CO), 169.38 (CH_3CO), 169.34 (CH_3CO), 142.28 (*C*$_{Ar}$), 129.62 (*C*H_{Ar}), 125,16 (*C*H_{Im}), 124.27 (*C*H_{Ar}), 122.21 (C$_{Ar}$), 120.13 (*C*H_{Im}), 87.72 (NC$H_{carbohidrate}$O), 73.57 (OC$H_{carbohidrate}CH_2$), 70.27 (*C*$H_{carbohidrate}$), 68.87 (*C*$H_{carbohidrate}$), 67.03 (*C*$H_{carbohidrate}$), 61.12 ($CHCH_{2carbohidrate}$OAc), 32.36 (*C*CH_3), 28.23 (C*C*H_3), 20.49 (COO*C*H_3), 20.41 (COO*C*H_3), 20.33 (COO*C*H_3), 20.29 (COO*C*H_3) ppm. MS (ESI): calcd. for $C_{58}H_{68}AgN_8O_{20}$: 1303.3575 (90), 1304.3607 (60), 1305.3583 (100), 1306.3605 (60), 1307.3630 (20); found: 1303.3595 (90), 1304.3626 (60), 1305.3605 (100), 1306.3629 (60), 1307.3655 (20).

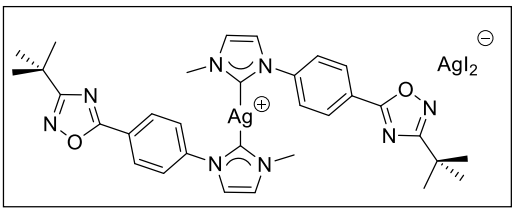
Synthesis of bis[1,3-di(4-(3-(*tert*-butyl)-1,2,4-oxadiazol)imidazolin-2-yliden]silver(I) dichloroargenate (74)



A solution of 1,3-bis(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole) imidazolium chloride (100 mg, 0.198 mmol) and Ag_2O (31.68 g, 0.138 mmol) in CH_3CN (4 mL) was stirred at 60°C for 24 h with exclusion of light. The suspension was filtered through Celite, and the solvent was concentrated under reduced pressure to ca. 0.5 mL and ethanol (2 ml) was added to give white-grey solid of bis[1,3-di(4-(3-(*tert*-butyl)-1,2,4-oxadiazol)imidazolin-2-yliden]silver(I) dichloroargenate. Yield*:* 93% (121.3 mg, 0.184 mmol).

IR (ATR): 1/λ = 3304, 3098, 2970, 2930, 2871, 1691, 1610, 1511, 1465, 1414, 1394, 1350, 1317, 1279, 1194, 1094, 1026, 991, 972, 944, 891, 844, 774, 735, 698, 606 cm^{-1}. ^1H NMR (300.1 MHz, DMSO-d_6): 8.51-8.37 (m, 8H, Ar-*H*), 8.34-8.07 (m, 12H, Ar-*H***,** C*H*$_{Im}$), 1.42 (s, 36H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, DMSO-d_6): δ = 179.01 (Ag*C*N), 178.02 (N*C*O), 173.54 (N*C*N), 137.65 (N*C*N), 135.53 (*C*$_{Ar}$), 129.75 (*C*H$_{Ar}$), 124.77 (*C*$_{Ar}$), 124.46 (*C*H$_{Ar}$), 122.89 (*C*H$_{Im}$), 32.20 (*C*(CH$_3$)$_3$), 28.14 (C(*C*H$_3$)$_3$) ppm. MS (ESI): 1043.48 (M^+-$AgCl_2$, 75), 1044.48 (M^+-$AgCl_2$, 45), 1045.37 (M^+-$AgCl_2$, 100), 1046.32 (M^+-$AgCl_2$, 45), 1047.30 (M^+-$AgCl_2$, 10), 469.2 (M^+, *NHC*).

Synthesis of bis[1-((4-(3-(*tert*-butyl)-1,2,4-oxadiazol)-3-methyl)imidazolin-2-yliden]-Ag(I) diiodoargenate (75)

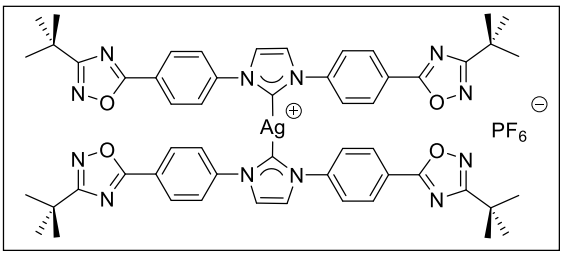


A solution of 1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-methyl-imidazolium iodide (81.23 mg, 0.198 mmol) and Ag_2O (31.68 g, 0.138 mmol) in CH_3CN (4 mL) was stirred at 60°C for 24 h with exclusion of light. The suspension was filtered through Celite, and the solvent was concentrated under reduced pressure to ca. 0.5 mL. Diethyl-ether (2 ml) was added in order to give pale-grey solid of bis[1-((4-(3-(*tert*-butyl)-1,2,4-oxadiazol)-3-methyl)imidazolin-2-yliden]silver(I) diiodoargenate. Yield*:* 73% (149.5 mg, 0.14 mmol).

IR (ATR): 1/λ = 3441, 3098, 2969, 3930, 2871, 1614, 1512, 1465, 1424, 1396, 1348, 1269, 1238, 1195, 1094, 1017, 991, 956, 892, 850, 774, 735, 695, 603, 547 cm^{-1}. UV/Vis (DCM): λmax. (lg ε) = 193 (1.084), 196 (1.078), 265 (0.678), 425 (0.021) nm. ^1H NMR (300.1 MHz,

DMSO-d_6): 8.08-8.03 (m, 4H, Ar-*H*), 7.99-7.93 (m, 6H, Ar-*H*, CH_{Im}), 7.76 (d, *J* = 1.8, 2H, CH_{Im}), 3.36 (s, 6H, CH_3), 1.39 (s, 18H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, DMSO-d_6): δ = 180.38 (CAg), 177.69 (N*C*O), 173.51 (N*C*N), 142.70 (C_{Ar}), 128.93 (CH$_{Ar}$), 124.56 (C_{Ar}), 124.36 (CH$_{Ar}$), 123.19 (CH$_{Im}$), 121.91 (CH$_{Im}$), 38.66 (C(CH$_3$)$_3$), 32.10 (NCH$_3$), 28.09 (C(CH3)$_3$) ppm. MS (ESI): 671.24 (M^+ - AgI_2, 100), 672.26 (M^+ - AgI_2, 30), 673.20 (M^+ - $AgCl_2$, 95), 674.18 (M^+ - $AgCl_2$, 20), 283.13 (M^+, NHC). $C_{32}H_{36}Ag_2I_2N_8O_2$ (1031.91): calcd. C 37.16, H 3.51, N 10.83; found C 37.04, H 3.68, N 11.71.

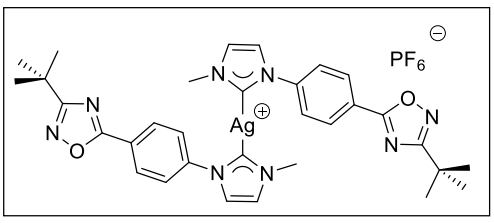
Synthesis of bis[1,3-di(4-(3-(*tert*-butyl)-1,2,4-oxadiazol)imidazolin-2-yliden]silver(I) hexafluorophosphate (76)



To a solution of bis[1,3-di(4-(3-(*tert*-butyl)-1,2,4-oxadiazol)imidazolin-2-yliden]-silver(I) dichloroargenate (**74**) (0.1 g, 0.08 mmol) in hot water (50 mL) was added a saturated aqueous solution of NH_4PF_6 (15 mL). The colorless precipitate which formed immediately was collected by filtration and washed with water. Yield*:* 95% (92 mg, 0.077 mmol).

IR (ATR): 1/λ = 3152, 2970, 2931, 2872, 1616, 1546, 1510, 1465, 1428, 1396, 1349, 1256, 1195, 1104, 1065, 1021, 992, 974, 951, 830, 774, 736, 696, 647, 607, 556 cm^{-1}. ^{1}H NMR (300.1 MHz, DMSO-d_6): 8.38-8.27 (m, 8H, Ar-*H*), 8.18-8.88 (m, 12H, Ar-*H*, CH_{im}), 1.43 (s, 36H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, DMSO-d_6): δ = 178.91 (Ag*C*N), 177.65 (N*C*O), 173.28 (N*C*N), 142.52 (N*C*N), 129.77 (C_{Ar}), 128.83 (CH$_{Ar}$), 124.54 (CH$_{Ar}$), 123.63 (C_{Ar}), 122.89 (CH$_{Im}$), 32.12 (C(CH$_3$)$_3$), 28.09 (C(CH$_3$)$_3$) ppm. MS (ESI): 1043.45 (M^+ - PF_6, 70), 1044.45 (M^+ - PF_6, 50), 1045.35 (M^+ - PF_6, 100), 1046.32 (M^+ - PF_6, 55), 1047.32 (M^+ - PF_6, 13), 469.2 (M^+, NHC).

Synthesis of Bis[1-((4-(3-(*tert*-butyl)-1,2,4-oxadiazol)-3-methyl)imidazolin-2-yliden]Ag(I) hexafluorophosphate (77)

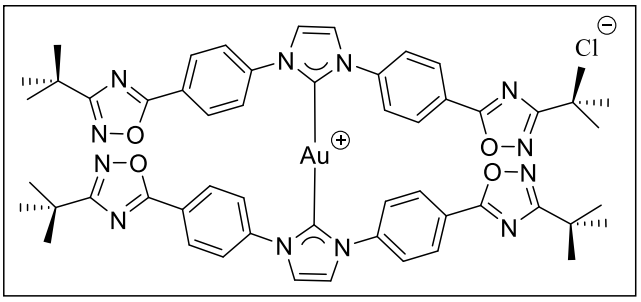


To a solution of bis[1-((4-(3-(*tert*-butyl)-1,2,4-oxadiazol)-3-methyl)imidazolin-2-yliden]silver(I) diiodoargenate (**75**) (0.1 g, 0.096 mmol) in hot water (50 mL) was added a saturated aqueous solution of

NH_4PF_6 (15 mL). The colorless precipitate which formed immediately was collected by filtration and washed with water. Yield*:* 90% (71 mg, 0.086 mmol)

IR (ATR): 1/λ = 3441, 3098, 2969, 3930, 2871, 1614, 1512, 1465, 1424, 1396, 1348, 1269, 1238, 1195, 1094, 1017, 991, 956, 892, 850, 774, 735, 695, 603, 547 cm^{-1}. UV/Vis (DCM): λmax. (lg ε) = 193 (1.084), 196 (1.078), 265 (0.678), 425 (0.021) nm. ^{1}H NMR (300.1 MHz, DMSO-d_6): 8.08-8.03 (m, 4H, Ar-*H*), 7.99-7.93 (m, 6H, Ar-*H***,** C*H*$_{Im}$), 7.76 (d, *J* = 1.8, 2H, *CH*$_{Im}$), 3.36 (s, 6H, C*H*$_3$), 1.39 (s, 18H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, DMSO-d_6): δ = 180.38 (*C*Ag), 177.69 (N*C*O), 173.51 (N*C*N), 142.70 (C_{Ar}), 128.93 (CH_{Ar}), 124.56 (C_{Ar}), 124.36 (CH_{Ar}), 123.19 (CH_{Im}), 121.91 (CH_{Im}), 38.66 ($C(CH_3)_3$), 32.10 (NCH_3), 28.09 ($C(CH_3)_3$) ppm. MS (ESI): 671.24 (M^+ - AgI_2, 100), 672.26 [M^+ - AgI_2, 30), 673.20 (M^+ - $AgCl_2$, 95), 674.18 (M^+ - $AgCl_2$, 20), 283.13 (M^+, NHC]. $C_{32}H_{36}Ag_2I_2N_8O_2$ (1031.91): calcd. C 37.16, H 3.51, N 10.83; found C 37.04, H 3.68, N 11.71.

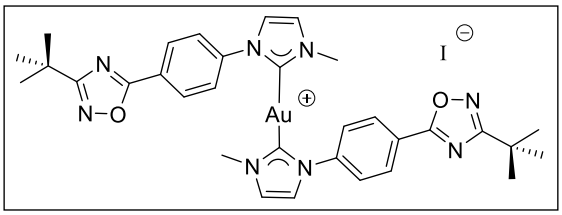
Synthesis of bis[1,3-di(4-(3-(*tert*-butyl)-1,2,4-oxadiazol)imidazolin-2-yliden]gold(I) chloride (78)



To a mixture of **74** (101 mg, 0.2 mmol) in acetone (5 mL), were added Me_2SAuCl (58.9 mg, 0.2 mmol) and K_2CO_3 (83 mg, 0.6 mmol). The resulting mixture was stirred for 24 h at 60 ºC in the absence of light. The solvent was removed in vacuum and the residue was flashed-chromatographed (DCM/Ethyl Acetate, 3:1). Yield*:* 56% (65 mg, 0.11 mmol).

IR (ATR): 1/λ = 3508, 3376, 3166, 2971, 2931, 1614, 1572, 1511, 1466, 1327, 1396, 1348, 1287, 1195, 1093, 1018, 991, 974, 944, 892, 850, 773, 751, 697, 611, 575, 544 cm^{-1}. ^{1}H NMR (300.1 MHz, $CDCl_3$): 8.09-8.01 (m, 8H, Ar-*H*), 7.94-7.82 (m, 8H, Ar-*H*), 7.73 (s, 4H, C*H*$_{Im}$), 1.46 (s, 36H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 184.3 (Au*C*N), 178.45 (N*C*O), 173.29 (N*C*N$_{ox}$), 141.36 (N*C*$_{Ar}$), 129.27 (*CH*$_{Ar}$), 125.44 (*CH*$_{Im}$), 32.58 ($C(CH_3)_3$), 28.43 ($C(CH_3)_3$) ppm. MS (HR-ESI): calcd. for $C_{54}H_{56}AuN_{12}O_4^+$: 1133.4207 (100), 1134.4239 (60), 1135.4269 (20), 1136.4300 (5); found: 1133.4216 (100), 1134.4237 (60), 1135.4262 (20), 1136.4295 (5).

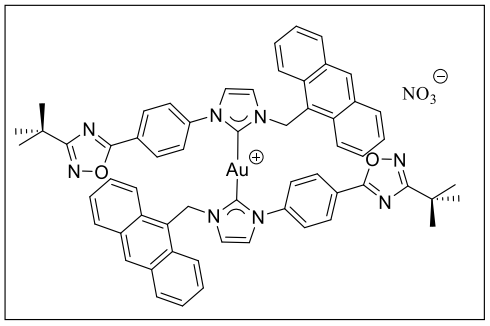
Synthesis of bis[1-((4-(3-(*tert*-butyl)-1,2,4-oxadiazol)-3-methyl)imidazolin-2-yliden]-gold(I) iodide (79)



To a mixture of **75** (82 mg, 0.2 mmol) in acetone (5 mL), were added Me_2SAuCl (58.9 mg, 0.2 mmol) and K_2CO_3 (83 mg, 0.6 mmol). The resulting mixture was stirred for 24 h at 60ºC in the absence of light. The solvent was removed in vacuo and the residue was flashed-chromatographed (DCM/Ethyl Acetate, 3:1). Yield*:* 43% (38.3 mg, 0.04 mmol).

IR (ATR): 1/λ = 3428, 3070, 2969, 2928, 2871, 1615, 1562, 1512, 1460, 1425, 1395, 1350, 1322, 1281, 1242, 1195, 1093, 1018, 991, 959, 891, 849, 774, 745, 698, 634, 603, 550 cm^{-1}. ^1H NMR (300.1 MHz, $CDCl_3$): 8.15-8.08 (m, 4H, Ar-*H*), 7.85-7.78 (m, 4H, Ar-*H*), 7.54 (d, J_{HH} = 1.9 Hz, 2H, CH_{Im}), 7.43 (d, J_{HH} = 1.9 Hz, 2H, CH_{Im}), 4.05 (s, 6 H, CH_3), 1.44 (s, 18H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 182.78 (Au*C*N), 178.43 (N*C*O), 173.43 (N*C*N_{ox}), 141.80 (NC_{Ar}), 129.10 (*C*H$_{Ar}$), 125.27 (*C*H$_{Ar}$), 125.01 (*C*H$_{Im}$), 124.38 (C_{Ar}), 121.87 (*C*H$_{Im}$), 39.19 (*C*H_3), 32.50 (*C*$(CH_3)_3$), 28.37 (C(*C*$H_3)_3$) ppm. MS (HR-ESI): calcd. for $C_{32}H_{36}AuN_8O_2^+$: 761.2627 (100), 762.2625 (40), 763.2678 (10); found: 761.2624 (100), 762.2648 (40), 763.2676 (10).

Synthesis of bis(1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(9-methyl-antracene)-imidazolium-2-ylidene) gold (I) nitrate (80)

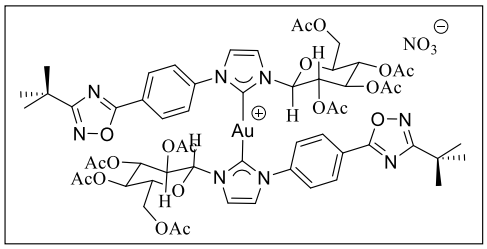


To a solution of bis(1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(9-methyl-antracene)-imidazolium-2-ylidene) silver(I) nitrate (0.1 g, 0.09 mmol) in dry CH_2Cl_2 (20 mL), AuCl(SMe_2) (27 mg, 0.09 mmol) was added and the mixture was stirred at room temperature for 12 h. A clear solution was obtained after filtration. The volatile components were removed under vacuum. The crude product was flash-chromatographed using DCM as eluent (r.f = 0.3). Yield*:* 73% (77 mg, 0.066 mmol).

IR (ATR): 1/λ = 3136, 3055, 2967, 2927, 2869, 1616, 1560, 1512, 1430, 1394, 1350, 1326, 1280, 1233, 1197, 1091, 1022, 958, 886, 847, 799, 774, 727, 706, 561, 630, 600, 577, 551 cm^{-1}. ^1H NMR (300.1 MHz, $CDCl_3$): δ = 8.60 (s, 2H, CH_{An}), 8.31-8.25 (m, 4H, CH_{An}), 8.09-8.03 (m, 4H, CH_{An}), 8.01-7.92 (m, 4H, Ar-*H*), 7.83-7.76 (m, 4H, Ar-*H*), 7.58-7.45 (m, 8H, CH_{An}),

7.41 (d, J_{HH} = 2 Hz, 2H, CH_{Im}), 6.94 (d, J_{HH} = 2 Hz, 2H, CH_{Im}), 6.32 (s, 4H, CH_2), 1.36 (s, 18H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 183.84 (Au*C*N), 179.13 (N*C*O), 174.53 (N*C*N_{ox}), 143.21 (NC_{Ar}), 132.34, 131.93, 130.04, 130.41, 129.91, 128.57, 126.70, 126.35, 125.79, 125.26, 124.27, 123.40 (CH$_{Im}$), 123.05 (CH$_{Im}$), 48.47 (CH$_2$-An), 33.13 (CCH$_3$), 28.69 (CCH$_3$) ppm. MS (HR-ESI): calcd. for $C_{60}H_{52}AuN_8O^+$: 1113.3879 (100), 1114.3904 (70), 1115.3935 (25), 1116.3965 (10); found: 1113.3879 (100), 1114.3901 (70), 1115.3927 (25), 1116.3957 (10).

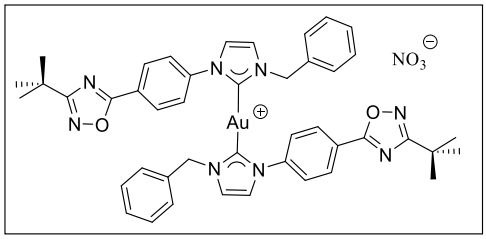
Synthesis of bis(1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(2,3,4,5-tetra-*O*-acetyl-*D*-glucopyranosyl)-imidazolium-2-ylidene) gold (I) nitrate (81)



To a solution of bis(1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(2,3,4,5-tetra-*O*-acetyl-*D*-glucopyranosyl)-imidazolium-2-ylidene) silver(I) nitrate (0.123 g, 0.09 mmol) in dry CH_2Cl_2 (20 mL), AuCl(SMe_2) (27 mg, 0.09 mmol) was added and the mixture was stirred at room temperature for 12 h. A clear solution was obtained after filtration. The volatile components were removed under vacuum. The crude product was flash-chromatographed using DCM:MeOH 10:1 as eluent (r.f = 0.4). Yield*:* 34% (44.5 mg, 0.03 mmol).

^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 8.12-8.06 (m, 4H, Ar-*H*), 7.76-7.68 (m, 6H, Ar-*H*, CH_{Im}), 7.59 (d, J_{HH} = 1.9 Hz, 2H, CH_{im}), 7.51 (d, J_{HH} = 1.9 Hz, 2H, CH_{im}), 6.63 (d, J_{HH} = 8.9 Hz, 2H, NC*H*O), 5.77-5.71 (m, 2H, 2 x C$H_{carbohidrate}$), 5.60 (d, J_{HH} = 2.9 Hz, 2H, CH_2), 5.52-5.41 (m, 2H, 2 x C$H_{carbohidrate}$), 4.59-4.48 (m, 2H, C$H_{carbohidrate}$), 4.28-4.16 (m, 4H, C$H_{carbohydrate}$, CH_2), 2.17 (s, 6H, CH_3), 2.05 (s, 6H, CH_3), 2.01 (s, 6H, CH_3), 1.81 (s, 6H, CH_3), 1.45 (s, 18H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 180.87 (Au*C*N),178.46 (N*C*O), 173.06 (N*C*N_{ox}), 170.41 (CH_3CO), 169.72 (CH_3CO), 169.47 (CH_3CO), 169.39 (CH_3CO), 141.44 (C_{Ar}), 129.25 (CH$_{Ar}$), 125.62 (CH$_{Im}$), 125.50 (CH$_{Ar}$), 123.41 (C$_{Ar}$), 120.23 (CH$_{Im}$), 86.60 (NCH$_{carbohidrate}$O), 73.53 (OCH$_{carbohidrate}$CH$_2$), 70.26 ($CH_{carbohidrate}$), 69.87 ($CH_{carbohidrate}$), 67.40 ($CH_{carbohidrate}$), 60.87 (CHCH$_{2carbohidrate}$OAc), 32.54 (CCH$_3$), 28.37 (CCH$_3$), 20.66 (COOCH$_3$), 20.56 (COOCH$_3$), 20.44 (COOCH$_3$), 20.41 (COOCH$_3$) ppm. MS (ESI): calcd. for $C_{58}H_{68}AuN_8O_{20}^+$: 1393.4187 (100), 1394.4207 (70), 1395.4230 (30), 1396.4255 (10); found: 1393.4209 (100), 1394.4241 (70), 1395.4269 (30), 1396.4296 (10).

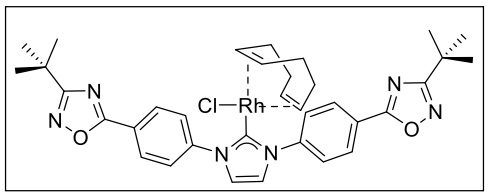
Synthesis of bis(1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(benzyl)-imidazolium-2-ylidene) gold (I) nitrate (82)



To a solution of bis(1-(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-(benzyl)-imidazolium-2-ylidene) silver (I) nitrate (0.08 g, 0.09 mmol) in dry CH_2Cl_2 (20 mL), $AuCl(SMe_2)$ (27 mg, 0.09 mmol) was added and the mixture was stirred at room temperature for 12 h. A clear solution was obtained after filtration. The volatile components were removed under vacuum. The crude product was flash-chromatographed using DCM : Methanol as eluent (r.f = 0.5). Yield*:* 43% (38 mg, 0.039 mmol).

^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 8.29-8.23 (m, 4H, Ar-*H*), 7.93-7.87 (m, 4H, Ar-*H*), 7.48-7.32 (m, 14H, C*H*$_{Im}$, Ar-*H*$_{Bn}$), 5.49 (s, 4H, C*H*$_2$), 1.44 (s, 18H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 182.39 (Au*C*N), 178.49 (N*C*O), 173.65 (N*C*N$_{ox}$), 141.85 (N*C*$_{Ar}$), 134.51, 129.42, 129.22, 129.12, 129.05, 128.86, 128.24, 127.36 (*C*H$_{Im}$), 125.28, 121.92 (*C*H$_{Im}$), 55.63 (*C*H$_2$-Bn), 32.48 (*C*CH$_3$), 28.36 (C*C*H$_3$). MS (HR-ESI): calcd. for $C_{44}H_{44}AuN_8O_2^+$: 913.3247 (100), 914.3277 (50), 915.3307 (15); found: 913.3237 (100), 914.3260 (50), 915.3288 (15).

Synthesis of Chloro(η^4-1,5-cyclooctadiene) (1,3-bis(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-1*H*-imidazol-2(3*H*)-ylidene)rhodium(I) (83)

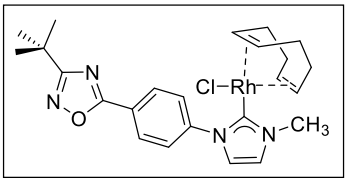


A mixture of 1,3-bis(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)imidazolium chloride (50.5 mg, 0.1 mmol) and silver(I) oxide (14 mg, 0.06 mmol) in dry dichloromethane (10 mL) was stirred for 24 h at room temperature in the absence of light. The mixture was filtered over Celite and $[Rh(COD)Cl]_2$ (25 mg, 0.05 mmol) was added. The resulting mixture was stirred for 12 h and filtered over Celite. The solvent was removed in vacuum and dichloromethane (2 mL) was added. The product was precipitated as a colorless solid upon addition of pentane and purified by column chromatography (DCM/Ethyl Acetate, 3:1). Yield*:* 59% (51 mg, 118 mmol).

IR (ATR): 1/λ = 3128, 2965, 2924, 2871, 2826, 1720, 1612, 1565, 1510, 1464, 1423, 1399, 1350, 1275, 1195, 1100, 1076, 1028, 992, 970, 935, 892, 845, 775, 724, 698, 636, 610, 575, 544 cm^{-1}. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 8.63-8.57 (m, 4H, Ar-*H*), 8.39-8.34 (m, 4H, Ar-*H*), 7.44 (s, 2H, C*H*), 5.13 (bs, 2H, COD$_{vinyl}$), 2.78 (bs, 2H, COD$_{vinyl}$), 2.01 (m, 4H, COD$_{allyl}$),

1.71 (m, 4H, COD_{allyl}), 1.45 (s, 18H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 185.9 (d, J_{RhC} = 52.4 Hz, *RhC*), 178.5, 173.73, 143.66, 128.74, 125.62, 125.42, 122.43, 96.4 (d, COD_{vinyl}), 96.4 (d, COD_{vinyl}), 68.9 (d, COD_{vinyl}), 33.0 (COD_{allyl}), 32.61, 29.8 (COD_{allyl}), 28.9 (COD_{allyl}), 28.45 ppm. MS (EI): 714.2 (25, M^+), 715.2 (15, M^+), 717.2 (5, M^+), 678.2 (30), 570.1 (20), 469.2 (100).

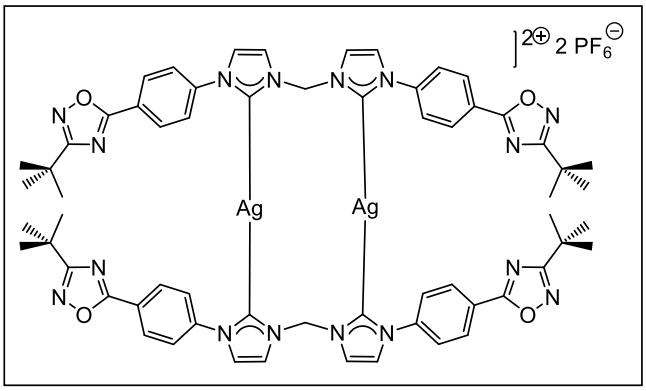
Synthesis of Chloro(η^4-1,5-cyclooctadiene) (1-(4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)-3-methyl-1*H*-imidazol-2(3*H*)-ylidene)rhodium(I) (84)



A mixture of 1-bis(3-*tert*-butyl-5-phenyl-1,2,4-oxadiazole)-3-methyl-imidazolium iodide (100 mg, 0.24 mmol) and silver(I) oxide (35 mg, 0.15 mmol) in dry dichloromethane (15 mL) was stirred for 24 h at room temperature in the absence of light. The mixture was filtered over Celite and $[Rh(COD)Cl]_2$ (59 mg, 0.12 mmol) was added. The resulting mixture was stirred for 12 h and filtered over Celite. The solvent was removed in vacuum and dichloromethane (2 mL) was added. The product was precipitated as a colorless solid upon addition of pentane and purified by column chromatography (DCM/Ethyl Acetate, 3:1). Yield: 44% (57 mg, 107 mmol).

IR (ATR): 1/λ = 3163, 3098, 2967, 2913, 2871, 2829, 1700, 1612, 1593, 1512, 1466, 1448, 1425, 1397, 1350, 1320, 1268, 1242, 1193, 1132, 1095, 1076, 991, 957, 887, 864, 846, 817, 775, 749, 697, 634, 603, 544 cm^{-1}. UV/Vis (DCM): λmax. (lg ε) = 227 (1.102), 264 (1.291), 395 (0.114) nm. ^1H NMR (300.1 MHz, $CDCl_3$): δ = 8.48-8.43 (m, 2H, Ar-*H*), 8.36-8.28 (m, 2H, Ar-*H*), 7.22 (d, *J* = 1.9, 1H, C*H*), 7.04 (d, *J* = 1.9, 1H, C*H*), 5.19-4.98 (m, 2H, COD_{vinyl}), 4.24 (s, 3H, CH_3), 3.29-3.23 (m, 1H, COD_{vinyl}), 2.58-2.51 (m, 1H, COD_{vinyl}), 2.41-2.32 (m, 2H, CH_2), 2.22-2.13 (m, 1H, CH_2), 1.91-1.73 (m, 4H, CH_2), 1.76-1.72 (m, 1H, CH_2), 1.46 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 184.35 (d, J_{RhC} = 51.29 Hz, Rh*C*), 178.50, 174.20, 143.19, 128.63, 124.78, 123.83, 123.20, 120.75, 98.20 (d, J_{RhC} = 7.08 Hz, Rh*C*), 78.70, 78.51, 68.25 (d, J_{RhC} = 14.53 Hz, Rh*C*), 60.31, 38.54, 33.15, 32.51, 31.88, 30.79, 28.88, 28.48, 28.45 ppm. MS (ESI): 528.1 ($M+H^+$), 493.1 (M-Cl); MS (EI): 528.1 (30, M^+), 530.1 (15, M^+), 531.1 (5, M^+), 492.1 (25), 384.1 (25), 283.1 (100). Anal. Calcd for $C_{24}H_{30}ClN_4ORh$: C, 54.5; H, 5.72; N, 10.59. Found: C, 53.95; H, 5.69; N, 10.25.

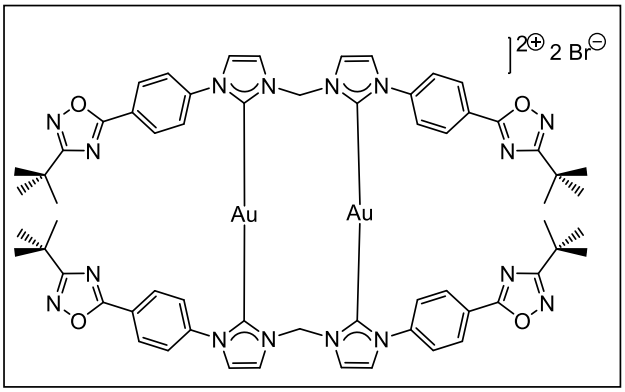
Bis {1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylene-diimidazolin-2,2′-diylidene}disilver(I) bis(hexafluorophosphate) (85)



A mixture of 1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylene-diimidazo-lium bis(hexafluorophosphate) (200 mg, 0.238 mmol) and Ag_2O (56 mg, 0.238 mmol) in CH_3CN (10 mL) was stirred for 48 h at 25°C under an inert atmosphere in the dark. The mixture was filtered over Celite and the solvent was removed in vacuum to yield a yellowish-white solid. The solid was dissolved in CH_2Cl_2, filtered, and concentrated to 1 mL under reduced pressure. Addition of Et_2O afforded a colorless solid which was filtered off and washed with ethyl acetate. Yield*:* 16% (90 mg, 0.068 mmol). Single crystals suitable for X-ray diffraction were obtained by slow evaporation of a concentrated solution of **85** in MeOH/DCM.

IR (ATR): 1/λ = 3144, 2971, 2931, 2873, 1616, 1512, 1463, 1425, 1395, 1351, 1249, 1196, 1104, 958, 827, 775, 735, 699, 603, 554 cm^{-1}. ^1H NMR (300.1 MHz, CD_3CN): 7.89 (d, J_{HH} = 1.9 Hz, 4H, NCHC*H*N), 7.76-7.67 (m, 12H, Ar-*H*, NC*H*CHN), 7.65-7.58 (m, 8H, Ar-*H*), 7.06 (bs, 2H, CH_2), 6.56 (bs, 2H, CH_2), 1.43 (s, 36H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, CD_3CN): δ = 188.1 (Ag*C*N), 179.2 (N*C*O), 174.4 (N*C*N_{ox}), 143.2 (NC_{Ar}), 129.9 (*C*H_{Ar}), 125.5 (C_{Ar}), 125.1 (*C*H_{Ar}), 124.3 (*C*H_{Im}), 123.7 (*C*H_{Im}), 66.2 (N*C*H_2N), 33.2 (*C*CH_3), 28.8 (C*C*H_3) ppm. MS (HR-ESI): calcd. for $C_{31}H_{32}AgN_8O_2$ (M^{2+}/2): 655.1693 (60), 655.6708 (40), 656.1695 (100), 656.6707 (70), 657.1698 (60), 657.6709 (40), 658.1721 (10); found: 655.1701 (60), 655.6716 (40), 656.1701 (100), 656.6709 (70), 657.1698 (70), 657.6707 (40), 658.1717 (10).

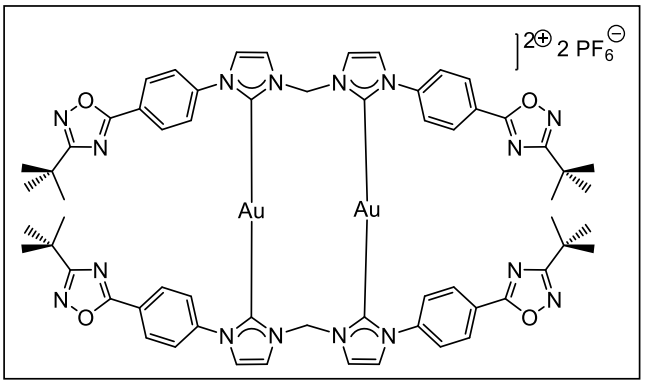
Bis{1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylene-diimidazolin-2,2′-diylidene}digold(I) bis(bromide) (86)



A suspension of 1,1′-[5-(phenyl)-3-(*tert* butyl)-1,2,4-oxadiazole]-3,3′-methylene-diimidazolium bis(bromide) (323.27 mg, 0.45 mmol) and (Me_2S)AuCl (134 mg, 0.48 mmol) in DMF (20 mL) was heated to 100 °C under an inert atmosphere. Sodium acetate (150 mg, 1.8 mmol) was added and the mixture was stirred at 100°C for 5 h, then cooled to room temperature and filtered. To the clear filtrate ether was added, yielding a colorless precipitate which was collected, washed with ether and dried. Yield*:* 40% (300 mg, 0.18 mmol).

IR (ATR): 1/λ = 3400, 3064, 2969, 2930, 2871, 1615, 1564, 1512, 1462, 1426, 1394, 1349, 1325, 1282, 1254, 1195, 1091, 1062, 1019, 991, 958, 892, 848, 774, 758, 736, 722, 698, 603, 551 cm^{-1}. ^{1}H NMR (300.1 MHz, DMSO-d_6): 8.59 (s, 4H, NCHC*H*N), 8.19 (s, 4H, NC*H*CHN), 7.99-7.51 (m, 16H, Ar-*H*), 6.92-6.68 (bs, 4H, C*H*2), 1.41 (s, 36H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, DMSO-d_6): δ = 181.6 (Au*C*N_{Im}), 177.5 (N*C*O), 173.1 (N*C*N_{ox}), 141.2 (N*C*$_{Ar}$), 128.4 (*C*H$_{Ar}$), 125.1 (*C*H$_{Ar}$), 124.1 (*C*$_{Ar}$), 123.5 (CH*C*HN_{Im}), 123.2 (*C*HCHN_{Im}), 63.1 (N*C*H_2N), 32.1 (*C*CH_3), 28.1 (C*CH*3) ppm. MS (HR-ESI): calcd. for $C_{31}H_{32}AuN_8O_2$ (M^{2+}/2) 745.2308 (100), 745.7322 (80), 746.2336 (30), 746.7350 (10); found 745.2315 (100), 745.7322 (80), 746.2332 (30), 746.7346 (10). Anal. Calcd for $C_{62}H_{64}Au_2Br_2N_{16}O_4.NH_4Br$: C, 42.58; H, 3.92; N, 13.61. Found: C, 42.25; H, 3.96; N, 13.88.

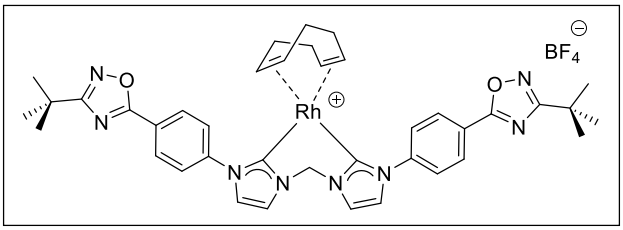
Bis{1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylene-diimidazolin-2,2′-diylidene}digold(I) bis(hexafluorophosphate) (87)



To a solution of **86** (0.1 g, 0.06 mmol) in hot water (50 mL) was added a saturated aqueous solution of NH_4PF_6 (15 mL). The colorless precipitate which formed immediately was collected by filtration and washed with water. Yield*:* 80% (86 mg, 0.048 mmol).

IR (ATR): 1/λ = 3187, 3158, 2970, 2930, 2872, 1616, 1512, 1463, 1427, 1396, 1375, 1349, 1326, 1260, 1193, 1093, 1022, 960, 833, 774, 731, 699, 603, 556 cm^{-1}. ^1H NMR (300.1 MHz, $CDCl_3$): 7.97 (s, 4H, NCHC*H*N), 7.88-7.76 (m, 8H, Ar-*H*), 7.62-49 (m, 8H, Ar-*H*), 7.45 (s, 4H, NC*H*CHN), 6.56-6.41 (bs, H, CH_2), 1.41 (s, 36H, *t*-Bu). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 182.3 (Au*C*N_{Im}), 178.4 (N*C*O), 173.4 (N*C*N_{ox}), 141.2 (NC_{Ar}), 129.1 (*C*H_{Ar}), 125.3 (*C*H_{Ar}), 124.9 (C_{Ar}), 123.4 (CH*C*HN_{Im}), 123.2 (*C*HCHN_{Im}), 63.2 (N*C*H_2N), 32.5 (*C*CH_3), 28.4 (C*C*H_3). MS (HR-ESI): calcd. for $C_{31}H_{32}AuN_8O_2$ (M^{2+}/2) 745.2308 (100), 745.7322 (80), 746.2336 (30), 746.7350 (10); found 745.2316 (100), 745.7323 (80), 746.2333 (30), 746.7347 (10). Anal. Calcd for $C_{62}H_{64}Au_2F_{12}N_{16}O_4P_2.C_4H_{10}O$: C, 42.73; H, 4.02; N, 12.08. Found: C, 42.92; H, 3.97; N, 12.48.

Synthesis of {1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylene-diimidazolin-2,2′-diylidene}Rh(η^2:η^2-COD)[BF_4] (88)

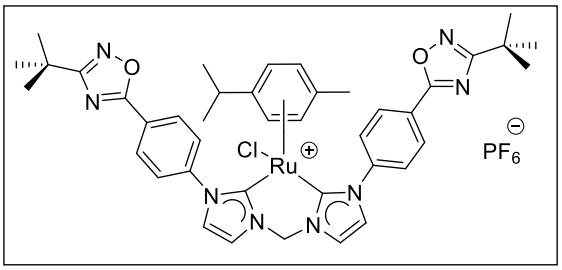


A suspension of **85**(BF_4 salt) (120 mg, 0.086 mmol) and $[Rh(COD)Cl]_2$ (43 mg, 0.086 mmol) was stirred in CH_2Cl_2 (15 mL) at 60°C for 5 h in the absence of light. The cooled reaction mixture was filtered, the solution was concentrated in vacuum to 1 mL and hexane was added to afford an orange solid. Yield*:* 81% (118 mg, 0.138 mmol).

IR (ATR): 1/λ = 3480, 3149, 2970, 2930, 2874, 2832, 1615, 1512, 1465, 1423, 1401, 1351, 1314, 1267, 1238, 1195, 1056, 851, 805, 774, 730, 697, 634, 604, 550 cm^{-1}. ^1H NMR (300.1 MHz, $CDCl_3$): 8.44-8.36 (m, 4H, Ar-*H*), 7.98-7.91 (m, 4H, Ar-*H*), 7.89 (d, *J* = 1.98 Hz, 2H,

NCH*CH*N), 7.29 (d, *J* = 1.98 Hz, 2H, N*CH*CHN), 6.73 (d, *J* = 12.9 Hz, 1H, CH_2), 6.52 (d, *J* = 12.9 Hz, 1H, CH_2), 4.59 (s, 2H, COD, *CH*), 3.59 (m, 2H, COD, *CH*), 2.14 (m, 2H, COD CH_2), 1.93 (m, 4H, COD, CH_2), 1.49 (s, 18H, *t*-Bu), 1.42 (m, 2H, COD, CH_2) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 179.50 (d, J_{RhC} = 53 Hz, Rh*C*), 178.68 (N*C*O), 173.71 (N*C*N_{ox}), 142.05 (NC_{Ar}), 129.20 (*C*H$_{Ar}$), 125.13 (*C*H$_{Ar}$), 125.11 (C_{Ar}), 123.59 (CH*CH*N_{Im}), 121.36 (*CH*CHN_{Im}), 93.58 (d, J_{RhC} = 8.0 Hz, COD, *C*H), 89.08 (d, J_{RhC} = 7.1 Hz, COD, C*H*), 63.76 (N*C*H_2N), 32.61 (*C*CH_3), 30.12 (COD, *C*H_2), 30.01 (COD, *C*H_2), 28.48 (C*C*H_3) ppm. MS (HR-ESI): Calcd. for $C_{39}H_{44}N_8O_2Rh$ (M^+): 759.2642 (100), 760.2676 (50), 761.2709 (15), 761.2709 (10). Found 759.2642 (100), 760.2666 (50), 761.2694 (10). Calcd. for $C_{31}H_{32}N_8O_2Rh$ (M^+-COD): 651.1703 (100), 652.1737 (30), 653.1770 (5). Found: 651.1701 (30), 652.1728 (10), 653.1767 (5).

Synthesis of RuCl(1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylenediimidazol) (η^6-*p*-cymene)]PF_6 (89)

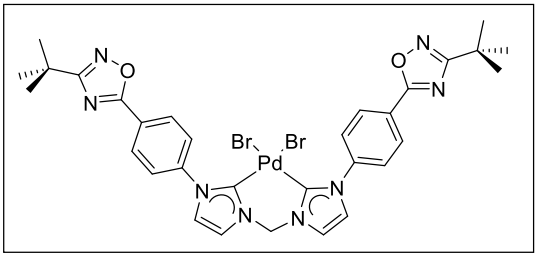


To a solution of 1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylene diimidazolium bis(bromide) (177.5 mg, 0.25 mmol) in H_2O (15 mL) was added Ag_2O (145 mg, 0.625 mmol). After stirring at room temperature in the absence of light for 60 min, the mixture was filtered over Celite. The clear filtrate was treated with NH_4PF_6 (325 mg, 2.0 mmol) and the colorless precipitate thus formed was collected and subsequently washed with H_2O and with Et_2O. The dry residue was dissolved in CH_2Cl_2 (10 mL) and [(η^6-*p*-cymene)-$RuCl_2$]$_2$ (75 mg, 125 mmol) was added. The reaction mixture was stirred for 24 hours at room temperature. Upon addition of Et_2O a precipitate as a brown solid was formed. The product was collected by filtration and dried under vacuum. Yield*:* 23% (56 mg, 0.057 mmol).

IR (ATR): 1/λ = 3494, 3439, 3098, 2973, 2933, 2873, 1615, 1574, 1512, 1465, 1425, 1398, 1348, 1272, 1242, 1196, 1106, 1090, 1016, 992, 956, 888, 849, 773, 695, 603, 551 cm^{-1}. ^{1}H NMR (300.1 MHz, DMSO-d_6): 8.33-8.24 (m, 4H, Ar-*H*), 8.14-8.05 (m, 4H, Ar-*H*), 7.89-7.81 (m, 4H, C*H*$_{Im}$), 6.63 (d, J_{HH} = 13.1 Hz, 1 H, NCH_2), 5.65 (d, J_{HH} = 13.1 Hz, 1H, NCH_2), 5.31-5.19 (m, 2H, $(CH_3)_2$CH-C_6H_4-(CH_3)-*p*), 4.89-4.78 (m, 2H, $(CH_3)_2$CH-C_6H_4-(CH_3)-*p*), 2.72 (m, 1H, $(CH_3)_2$C*H*$C_6H_4(CH_3)$-*p*), 2.09 (s, 3H, $(CH_3)_2CHC_6H_4$-(CH_3)-*p*), 1.41 (s, 18H, *t*-Bu), 0.76 (d, J_{HH} = 6.9 Hz, 6H, *(*CH_3*)*$_2$$CHC_6H_4(CH_3)$-*p*) ppm. ^{13}C NMR (75.47 MHz, DMSO-d_6): δ = 179.23 (N*C*O), 176.53 (Ru*C*), 174.34 (N*C*N_{ox}), 143.65 (NC_{Ar}), 129.71 (*CH*$_{Ar}$), 128.47

(*C*$_{Ar}$), 125.76 (CH*C*HN_{Im}), 124.44 (*C*H_{Ar}), 122.63 (*C*HCHN_{Im}), 110.89 (*C*H_{Cym}), 106.45 (*C*H_{Cym}), 91.93 (*C*$_{Cym}$), 86.72 (*C*$_{Cym}$), 66.16 (N*C*H_2N), 33.27 (*C*CH_3), 31.34 (*C*H_{3Cym}), 28.48 (C*C*H_3), 22.7 (*C*H_{iPr}), 18.7 (*C*H_{3iPr}) ppm. MS (HR-ESI): calculated for $C_{41}H_{46}ClN_8O_2Ru^+$: 819.2476; found: 819.2479.

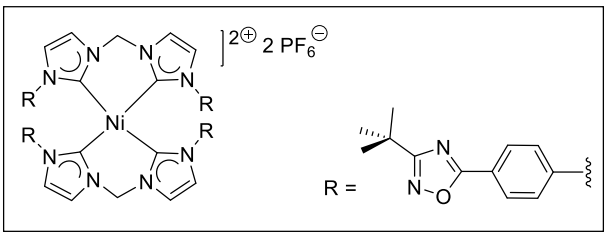
Synthesis of 1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylenediimidazolium palladium(II) dibromide (90)



A suspension of 1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylenediimidazo-lium bis-bromide (100 mg, 0.140 mmol) and $Pd(OAc)_2$ (16 mg, 0.071 mmol) was stirred in CH_3CN (25 mL) at 60°C for 12 hours. The reaction mixture was cooled to room temperature and the colorless precipitate thus formed was filtered and dried. Yield*:* 67% (77 mg, 0.094 mmol).

IR (ATR): 1/λ = 3491, 3086, 2968, 2928, 2871, 1616, 1571, 1512, 1466, 1429, 1412, 1395, 1352, 1316, 1279, 1239, 1194, 1129, 1094, 1023, 993, 959, 896, 846, 802, 775, 730, 698, 683, 672, 634, 603, 542 cm^{-1}. ^{1}H-NMR (300.1 MHz, DMSO-d_6): 8.48-8.36 (m, 4H, Ar-*H*), 8.17-8.06 (m, 4H, Ar-*H*), 8.03-7.89 (m, 4H, C*H*$_{im}$), 6.68-6.49 (m, 2H, C*H*$_2$), 1.44 (s, 18H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, DMSO-d_6): δ = 178.03 (Pd*C*N), 177.94 (N*C*O), 174.88 (N*C*N_{ox}), 143.49 (N*C*$_{Ar}$), 129.92 (*C*H_{Ar}), 128.17 (*C*$_{Ar}$), 124.91 (*C*H_{Im}), 122.94 (*C*H_{Ar}), 121.56 (*C*H_{Im}), 65.42 (N*C*H_2N), 32.19 (*C*CH_3), 28.14 (C*C*H_3) ppm. MS (HR-ESI): calcd. for $C_{31}H_{31}BrN_8O_2Pd$ (M-HBr): 732.0788 (100), 733.9542 (100); found: 732.1633 (100), 733.9644 (100).

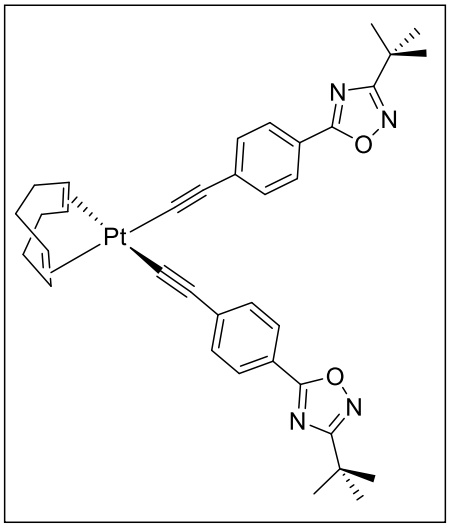
Synthesis of 1,1′-[5-(phenyl)-3-(*tert*-butyl)-1,2,4-oxadiazole]-3,3′-methylenediimidazolium nickel(II) bis(hexafluorophosphate) (91)



A suspension of 1,1′-[5-(phenyl)-3-(*tert* butyl)-1,2,4-oxadiazole]-3,3′-methylenediimi-dazolium di-silver(I) bis(hexafluorophospha-te) (100 mg, 0.062 mmol) and $Ni(PPh_3)_2Cl_2$ (89 mg, 0.125 mmol) was stirred in DCM (25 mL) at room temperature for 12 hours. The precipitate thus formed was filtered and the solvent was removed. The product was recrystallized from MeOH/ether.

MS-ESI: calcd. for $C_{62}H_{64}N_{16}O_4Ni$ (M^+): 1154.46; found: 1154.44. MS-ESI: calcd. for $C_{31}H_{32}N_8O_2Ni$ (M^{2+}+Ni): 606.20; found: 606.33. MS-ESI: calcd. for $C_{31}H_{32}N_8O_2Ni$ (M^{2+}): 548.26; found: 548.20.3.

Synthesis of {(COD)Pt[3-(*tert*-butyl)-5-(4-ethynylphenyl)-1,2,4-oxadiazole]$_2$} (92)

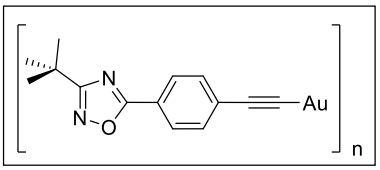


A suspension of 100 mg (0.267 mmol) $[(COD)PtCl_2]$ in 10 mL of ethanol was cooled at -5°C. A freshly prepared mixture of 132.82 mg of 3-(*tert*-butyl)-5-(4-ethynylphenyl)-1,2,4-oxadiazole (0.587 mmol, 2.2 eq.) and 66 mg (0.587 mmol, 2.2 eq.) of potassium *tert*-butoxide in 5 mL of ethanol were added dropwise with constant stirring. The solution changed color (became darker), and after 1 h the solid thus formed was filtered off. Recrystallization from CH_2Cl_2/hexane gave the pure product as microcrystalline material. Yield*:* 54% (108.7 mg, 0.144 mmol).

IR (ATR): $1/\lambda$ = 3049, 2968, 2928, 2904, 2872, 2124, 1606, 1580, 1547, 1509, 1464, 1411, 1395, 1350, 1313, 1270, 1195, 1178, 1093, 995, 970, 886, 848, 775, 739, 699, 653, 591 cm^{-1}. UV/Vis (DCM): λmax. (lg ε) = 221 (0.555), 229 (0.811), 303 (1.799), 480 (0.040) nm. ^1H NMR (300.1 MHz, $CDCl_3$): 8.04-7.97 (m, 4H, Ar-*H*), 7.55-7.48 (m, 4H, Ar-*H*), 5.91-5.62 (m, 4H, COD, C*H*, J_{PtH} = 43 Hz), 2.71-2.53 (m, 8H, COD, CH_2), 1.42 (s, 18H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 178.34, 175.2, 132.23, 131.02, 127.61, 122.4, 108.23, 105.05,

99.12, 32.45, 30.41, 28.48 ppm. MS (HR-ESI): calcd. for $C_{36}H_{38}N_4O_2PtH^+$ (M + H^+) 754.2721, found 754.2719; $C_{36}H_{38}N_4O_2PtK^+$ (M + K^+) 792.2280 found 792.2279.

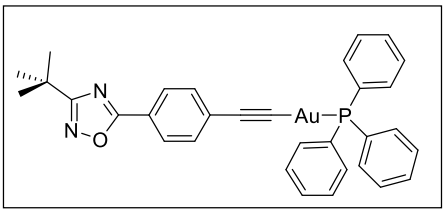
Synthesis of ((4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)ethynyl)gold polimer (93)



To a solution of 398.3 mg of 3-tert-butyl-5-(4-ethynylphenyl)-1,2,4-oxadiazole (1.76 mmol) in CH_2Cl_2 (20 mL) were successively added NEt_3 (1 mL) and 519 mg [$AuCl(SMe_2)$], (1.76 mmol). The reaction mixture was stirred for 1 h. The resulting solution was concentrated under reduced pressure to ca. 10 mL and MeOH (10 mL) was added to precipitate a yellow-green powder which was filtered, washed with Et_2O (2 x 5 mL) and dried. Yield 71% (530 mg, 1.25 mmol).

IR (ATR): 1/λ = 2968, 2929, 2870, 1985, 1609, 1581, 1549, 1505, 1463, 1407, 1394, 1347, 1270, 1194, 1092, 1016, 989, 971, 896, 841, 772, 735, 695, 650, 589 cm^{-1}. Anal. Calc. for C14H13AuN2O: C 39.82; H 3.10; N 6.63. Found: C, 39.59; H 3.09; N, 6.61%.

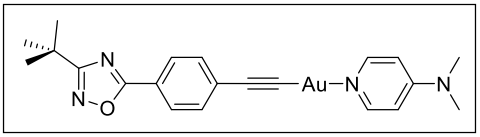
Synthesis of ((4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)ethynyl)gold(I)-PPh_3 (94)



To a suspension of 223.6 mg ((4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)ethynyl)gold polymer (0.53 mmol) in CH_2Cl_2 (25 mL) was added 209 mg of PPh_3 (0.8 mmol). The reaction mixture was stirred for 1h and filtered through anhydrous $MgSO_4$. The solution was concentrated under vacuum to 1 mL and hexane (10 mL) was added. After stirring for 15 hours, the suspension was filtered and the solid was air dried to give a colorless solid. Yield 66% (240 mg, 0.35mmol).

^{1}H NMR (300.1 MHz, $CDCl_3$): 8.06-7.99 (m, 2H, Ar-*H*), 7.65-7.41 (m, 17H, Ar-*H*, P*Ph*$_3$), 1.43 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 178.26, 174.92, 134.35, 134.16, 132.73, 131.61, 131.58, 129.96, 129.32, 129.24, 129.09, 127.70, 122.39, 103.35, 32.44, 28.45 ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 42.13 (s, *P*Ph_3) ppm. MS (ESI): 721.18 (M + K^+, 100), 722.14 (M + K^+, 40), 723.11 (M + K^+, 8).

Synthesis of ((4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)phenyl)ethynyl)gold(I)-dimethylaminopyridine complex (95)

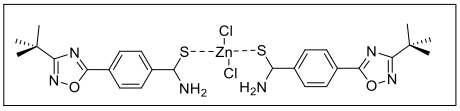


To a suspension of 100 mg ((4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)phenyl)ethynyl)-gold(I) polymer (0.183 mmol) in CH_2Cl_2 (5 mL) was added 22.44 mg of

DMAP (0.183 mmol). The reaction mixture was stirred for 2h and filtered through anhydrous Celite. The solution was concentrated under vacuum to 1 mL and Et_2O (10 mL) was added. After stirring for 15 hours, the suspension was filtered and the solid was air dried to give a colorless solid. Yield 53% (52.7 mg, 0.096mmol).

^{1}H NMR (300.1 MHz, $CDCl_3$): 8.07-7.95 (m, 4H, Py-*H*, Ar-*H*), 7.62-7.53 (m, 2H, Ar-*H*), 6.62-6.51 (m, 2H, Py-*H*), 3.07 (s, 6H, C*H*$_3$), 1.42 (s, 9H, *t*-Bu). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 178.24, 174.97, 154.76, 150.12, 132.77, 127.60, 107.42, 39.33, 32.43, 28.46. MS (HR-ESI): calcd. for $C_{36}H_{38}N_4O_2PtH^+$ (M + H^+) 754.2721, found 754.2719; $C_{21}H_{23}AuN_4Na^+$ (M + Na^+) 567.1429 (100), 568.1459 (20), 569.1488 (5); found 567.1430 (100), 568.1462 (20), 569.1495 (5).

Synthesis of {Zn[4-(3-*tert*-butyl-1,2,4-oxadiazol-5-yl)benzothioamide)]$_2$Cl$_2$} (96)



A solution of 300 mg of 4-(3-tert-butyl-1,2,4-oxadiazol-5-yl)benzothioamide (1.14 mmol) and 82 mg of $ZnCl_2$ (0.6 mmol) in absolut ethanol (10 mL) was refluxed for 48 hours under inert atmosphere. After cooling, the precipitate thus formed was filtrated and washed with 5 mL ethanol and then was dried in vacuum to afford 96 mg of powdery colorless solid. Yield: 24% (96 mg, 0.145 mmol).

VIII. Crystallographic data

Compound	**1**	**3**	**4**
Identification code	cmx6	cmx7	cmx12
Empirical formula	$C_{12}H_{13}IN_2O$	$C_{12}H_{15}N_3O$	$C_{12}H_{13}N_3O_3$
Formula weight	328.14	217.27	247.25
Crystal system	Monoclinic	Monoclinic	Orthorhombic
Space group	$P2_1/c$	$P2_1/c$	*Pbca*
Crystal size [mm]	0.17 x 0.17 x 0.06	0.15 x 0.15 x 0.1	0.24 x 0.12 x 0.07
a (Å)	14.2079(4)	15.6514(10)	6.7175(4)
b (Å)	6.6247(2)	9.0961(6)	13.8116(6)
c (Å)	14.2917(4)	18.4999(12)	26.1018(9)
Volume (Å^3)	1257.67(6)	2399.1(3)	2421.7(2)
α (°)	90	90	90
β (°)	110.781(2)	114.371(8)	90
δ (°)	90	90	90
Z	4	8	8
Density (calc.) [g/cm-3]	1.733	1.203	1.356
μ [mm^{-1}]	2.528	0.08	0.10
Temperature [K]	100(2)	100(2)	100(2)
Wavelength [Å]	0.71073	0.71073	0.71073
Theta range [°]	2.89 to 26.37	2.24 to 26.37	2.95 to 27.48
Reflections collected	38888	41755	55696
Independent reflections	2574	4908	2483
R1 with $I_0>2\sigma(I_0)$	0.0154	0.035	0.031
*w*R2	0.0388	0.057	0.061
G_0F	1.057	0.73	0.81
Largest diff. peak and hole [e/Å^3]	0.473 and -0.237	0.165 and -0.187	0.195 and -0.159
Structure solved by	Daniliuc Constantin	Daniliuc Constantin	Daniliuc Constantin

Compound	**5**	**7**	**8**
Identification code	cmx1	cmx5	cmx3
Empirical formula	$C_{13}H_{13}N_3O$	$C_{13}H_{14}N_2O_2$	$C_{14}H_{16}N_2O_2$
Formula weight	227.26	230.26	244.29
Crystal system	Monoclinic	Monoclinic	Triclinic
Space group	$P2_1/m$	$P2_1/m$	P-1
Crystal size [mm]	0.31 x 0.25 x 0.17	0.25 x 0.22 x 0.13	0.10 x 0.04 x 0.04
a (Å)	9.1200(4)	9.2714(2)	7.0155(6)
b (Å)	6.7180(4)	6.7055(2)	12.9272(10)
c (Å)	9.4957(4)	9.6182(2)	15.9808(12)
Volume (Å^3)	575.75(5)	592.58(3)	1293.93(18)
α (°)	90	90	113.416(8)
β (°)	98.259(4)	97.689(2)	101.242(6)
δ (°)	90	90	92.092(6)
Z	2	2	4
Density (calc.) [g/cm-3]	1.311	1.290	1.254
μ [mm^{-1}]	0.086	0.089	0.687
Temperature [K]	100(2)	100(2)	100(2)
Wavelength [Å]	0.71073	0.71073	1.54184
Theta range [°]	2.17 to 29.13	2.87 to 30.49	3.75 to 75.99
Reflections collected	15358	29178	21903
Independent reflections	1842	1949	5348
R1 with $I_0>2\sigma(I_0)$	0.0365	0.0363	0.0374
wR2	0.1068	0.1103	0.1018
G_0F	1.07	1.089	1.035
Largest diff. peak and hole [e/Å^3]	0.349 and -0.263	0.456 and -0.234	0.172 and -0.244
Structure solved by	Daniliuc Constantin	Daniliuc Constantin	Daniliuc Constantin

Compound	**13**	**18**	**19**
Identification code	cmx47	cmx99	cmx16
Empirical formula	$C_{14}H_{13}BrN_2O$	$C_{13}H_{15}N_3OS$	$C_{16}H_{21}N_3O_3$
Formula weight	305.17	261.34	303.36
Crystal system	Orthorhombic	Triclinic	Triclinic
Space group	P n a 2_1	*P*-1	P-1
Crystal size [mm]	0.34 x 0.29 x 0.14	0.11 x 0.07 x 0.06	0.27 x 0.18 x 0.13
a (Å)	22.5232(4)	5.5204(4)	8.2544(4)
b (Å)	8.4735(2)	10.3978(8)	10.0923(4)
c (Å)	7.0442(2)	11.3847(8)	10.6207(6)
Volume (Å^3)	1344.39(6)	643.80(8)	807.27(7)
α (°)	90	93.567(6)	105.700(4)
β (°)	90	93.498(6)	93.484(4)
δ (°)	90	98.277(6)	106.559(4)
Z	4	2	2
Density (calc.) [g/cm-3]	1.508	1.348	1.248
μ [mm^{-1}]	3.047	0.243	0.088
Temperature [K]	100(2)	100(2)	100(2)
Wavelength [Å]	0.71073	0.71073	0.71073
Theta range [°]	2.57 to 29.13	2.58 to 27.10	2.21 to 27.87
Reflections collected	57174	24223	27623
Independent reflections	3627	2824	3856
R1 with $I_0>2\sigma(I_0)$	0.0188	0.0347	0.0339
*w*R2	0.0489	0.0798	0.0969
G_0F	1.011	1.059	1.063
Largest diff. peak and hole [e/Å^3]	0.298 and -0.653	0.359 and -0.237	0.288 and -0.304
Structure solved by	Daniliuc Constantin	Daniliuc Constantin	Daniliuc Constantin

Compound	**20**	**24**	**26**
Identification code	cmx17	cmx31	cmx55
Empirical formula	$C_{12}H_{15}N_3O_2$	$C_{17}H_{23}N_3O_3$	$C_{16}H_{17}N_3O_3$
Formula weight	233.27	317.38	299.33
Crystal system	Orthorhombic	Monoclinic	Monoclinic
Space group	$P2_12_12_1$	$P2_1/c$	$P2_1/c$
Crystal size [mm]	0.19 x 0.19 x 0.16	0.19 x 0.04 x 0.04	0.11 x 0.05 x 0.02
a (Å)	10.3093(2)	9.8062(2)	13.8569(4)
b (Å)	10.4887(2)	17.6339(4)	15.3562(4)
c (Å)	11.3309(4)	10.0402(2)	6.8926(2)
Volume (Å^3)	1225.22(5)	1735.09(6)	1461.27(7)
α (°)	90	90	90
β (°)	90	92.022(2)	94.918(2)
δ (°)	90	90	90
Z	4	4	4
Density (calc.) [g/cm-3]	1.265	1.215	1.361
μ [mm^{-1}]	0.089	0.686	0.789
Temperature [K]	100(2)	100(2)	100(2)
Wavelength [Å]	0.71073	1.54184	1.54184
Theta range [°]	2.65 to 29.12	4.51 to 76.13	3.20 to 75.96
Reflections collected	53131	26793	16680
Independent reflections	1888	3591	3034
R1 with $I_0>2\sigma(I_0)$	0.0289	0.0356	0.0323
*w*R2	0.0772	0.0943	0.086
G_0F	1.062	1.055	1.06
Largest diff. peak and hole [e/Å^3]	0.261 and -0.160	0.193 and -0.226	0.210 and -0.190
Structure solved by	Daniliuc Constantin	Daniliuc Constantin	Daniliuc Constantin

Compound	**27**	**28**	**31**
Identification code	cmx53_twin5	cmx52	cmx10
Empirical formula	$C_{17}H_{21}N_3O_4$	$C_{16}H_{17}N_3O_4$	$C_{16}H_{20}N_4O_2$
Formula weight	331.37	315.33	300.36
Crystal system	Monoclinic	Trigonal	Triclinic
Space group	*P*(-1)	*R*(-3)	*P*-1
Crystal size [mm]	0.18 x 0.09 x 0.02	0.07 x 0.05 x 0.03	0.18 x 0.09 x 0.04
a (Å)	5.4494(5)	28.0192(16)	7.1791(6)
b (Å)	6.1403(6)	28.0192(16)	9.5004(8)
c (Å)	25.283(2)	10.0740(8)	23.8791(9)
Volume (Å^3)	805.57(13)	6849.3(8)	1617.7(2)
α (°)	86.906(8)	90	95.138(6)
β (°)	85.277(8)	90	94.122(6)
δ (°)	72.926(8)	120	90.648(6)
Z	2	18	4
Density (calc.) [g/cm-3]	1.366	1.376	1.233
μ [mm^{-1}]	0.814	0.837	0.084
Temperature [K]	100(2)	100(2)	100(2)
Wavelength [Å]	1.54184	1.54184	0.71073
Theta range [°]	3.51 to 76.03	3.15 to 75.91	2.15 to 26.37
Reflections collected	3747	28530	44110
Independent reflections	3747	3156	6586
R1 with $I_0>2\sigma(I_0)$	0.039	0.0385	0.0488
*w*R2	0.098	0.0923	0.1012
G_0F	1.04	1.039	0.721
Largest diff. peak and hole [e/Å^3]	0.377 and -0.339	0.174 and -0.219	0.507 and -0.248
Structure solved by	Daniliuc Constantin	Daniliuc Constantin	Daniliuc Constantin

Compound	**33**	**35**	**37**
Identification code	cmx9a	cmx89	cmx24
Empirical formula	$C_{14}H_{16}N_2O_3$	$C_{17}H_{21}N_3O_2$	$C_{14}H_{16}N_2O_3$
Formula weight	260.29	299.37	260.29
Crystal system	Monoclinic	Monoclinic	Orthorhombic
Space group	$P2_1/m$	$P2_1/c$	$Pbcn$
Crystal size [mm]	0.11 x 0.09 x 0.06	0.14 x 0.12 x 0.04	0.24 x 0.16 x 0.03
a (Å)	7.6876(6)	5.4582(4)	24.459(5)
b (Å)	6.5688(6)	11.0023(6)	12.310(3)
c (Å)	13.3016(10)	26.3293(16)	8.9524(18)
Volume (Å^3)	666.89(9)	1571.93(17)	2695.4(9)
α (°)	90	90	90
β (°)	96.867(8)	96.191(6)	90
δ (°)	90	90	90
Z	2	4	8
Density (calc.) [g/cm-3]	1.296	1.265	1.283
μ [mm^{-1}]	0.092	0.679	0.091
Temperature [K]	100(2)	100(2)	100(2)
Wavelength [Å]	0.71073	1.54184	0.71073
Theta range [°]	2.67 to 27.48	3.38 to 74.47	2.93 to 27.88
Reflections collected	24572	20138	76664
Independent reflections	1493	3266	3204
R1 with $I_0>2\sigma(I_0)$	0.033	0.034	0.032
wR2	0.068	0.092	0.0565
G_0F	0.82	1.05	0.802
Largest diff. peak and hole [e/Å^3]	0.196 and -0.236	0.221 and -0.185	0.165 and -0.205
Structure solved by	Daniliuc Constantin	Daniliuc Constantin	Daniliuc Constantin

Compound	**39**	**40**	**41**
Identification code	cmx87	cmx87a	cmx43
Empirical formula	$C_{17}H_{18}N_6OS$	$C_{19}H_{20}N_6O_2S$	$C_{13}H_{13}N_3OS$
Formula weight	354.43	396.47	259.32
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	*C*2/*c*	$P2_1/n$	$P2_1/n$
Crystal size [mm]	0.05 x 0.03 x 0.03	0.14 x 0.08 x 0.06	0.05 x 0.05 x 0.03
a (Å)	16.1767(4)	5.1644(4)	14.9371(4)
b (Å)	14.8235(4)	12.2462(8)	6.4523(2)
c (Å)	14.6649(4)	30.864(2)	15.5069(4)
Volume (Å^3)	3429.24(16)	1947.6(2)	1317.51(6)
α (°)	90	90	90
β (°)	102.796(2)	93.867(6)	118.170(4)
δ (°)	90	90	90
Z	8	4	4
Density (calc.) [g/cm-3]	1.373	1.352	1.307
μ [mm^{-1}]	1.832	1.712	2.116
Temperature [K]	100(2)	100(2)	100(2)
Wavelength [Å]	1.54184	1.54184	1.54184
Theta range [°]	4.09 to 76.10	3.88 to 74.48	3.39 to 76.01
Reflections collected	26821	16881	17130
Independent reflections	3571	4039	2710
R1 with $I_0>2\sigma(I_0)$	0.0478	0.038	0.033
*w*R2	0.1313	0.096	0.096
G_0F	1.05	1.02	1.09
Largest diff. peak and hole [e/Å^3]	0.600 and -0.752	0.268 and -0.327	0.408 and -0.407
Structure solved by	Daniliuc Constantin	Daniliuc Constantin	Daniliuc Constantin

Compound	**42**	**43 . CH_3OH**	**47 . 5/3DMSO**
Identification code	cmx73	cmx76	cmx78
Empirical formula	$C_{17}H_{17}N_5OS$	$C_{17}H_{21}N_7O_2$	$C_{26.33}H_{32}N_8O_{2.67}S_{1.67}$
Formula weight	339.42	355.41	556.70
Crystal system	Triclinic	Monoclinic	Triclinic
Space group	*P*-1	$P2_1/n$	*P*-1
Crystal size [mm]	0.28 x 0.20 x 0.03	0.26 x 0.20 x 0.14	0.17 x 0.10 x 0.02
a (Å)	8.8850(4)	7.5681(2)	10.5098(6)
b (Å)	14.5822(8)	15.8937(4)	17.6996(14)
c (Å)	14.6106(10)	16.0537(4)	23.2169(16)
Volume ($Å^3$)	1754.05(17)	1915.66(8)	4145.7(5)
α (°)	111.830(6)	90	75.968(6)
β (°)	92.985(4)	97.231(2)	82.825(6)
δ (°)	90.506(4)	90	84.179(6)
Z	4	4	6
Density (calc.) [g/cm-3]	1.285	1.232	1.338
μ [mm^{-1}]	0.198	0.086	1.862
Temperature [K]	100(2)	100(2)	100(2)
Wavelength [Å]	0.71073	0.71073	1.54184
Theta range [°]	2.30 to 27.48	2.56 to 26.37	3.60 to 75.95
Reflections collected	59195	54249	48006
Independent reflections	7162	3906	17122
R1 with $I_0>2\sigma(I_0)$	0.039	0.049	0.0556
*w*R2	0.072	0.09	0.147
G_0F	0.76	0.94	0.923
Largest diff. peak and hole [$e/Å^3$]	0.35 and -0.25	0.191 and -0.184	0.735 and -0.732
Structure solved by	Daniliuc Constantin	Daniliuc Constantin	Daniliuc Constantin

Compound	**58 . 2CHCl$_3$**	**59**	**60**
Identification code	ict03	ict05	ict21
Empirical formula	$C_{29}H_{30}AuCl_7N_6O_2$	$C_{16}H_{18}AuClN_4O$	$C_{22}H_{22}AuBrN_4O$
Formula weight	939.71	514.76	635.31
Crystal system	Triclinic	Monoclinic	Monoclinic
Space group	*P*-1	$P2_1/c$	$P2_1/c$
Crystal size [mm]	0.24 x 0.06 x 0.02	0.20 x 0.08 x 0.04	0.18 x 0.18 x 0.10
a (Å)	9.4854(3)	13.1177(5)	26.501(2)
b (Å)	13.2761(5)	7.5040(3)	13.3853(10)
c (Å)	15.1158(6)	17.5691(8)	12.3462(11)
Volume (Å^3)	1772.41(11)	1701.72(12)	4379.5(6)
α (°)	110.499(3)	90	90
β (°)	93.416(3)	100.270(4)	90.277(8)
δ (°)	93.686(3)	90	90
Z	2	4	8
Density (calc.) [g/cm-3]	1.761	2.009	1.927
μ [mm^{-1}]	4.715	8.811	8.6
Temperature [K]	293(2)	100(2)	100(2)
Wavelength [Å]	0.71073	0.71073	0.71073
Theta range [°]	2.16 to 27.88	2.36 to 30.87	2.16 to 28.28
Reflections collected	76226	83443	81844
Independent reflections	8426	5200	10871
R1 with $I_0>2\sigma(I_0)$	0.0314	0.0289	0.0382
*w*R2	0.0655	0.0415	0.0811
G_0F	1.034	1.069	1.03
Largest diff. peak and hole [e/Å^3]	2.213 and -1.626	2.901 and -0.804	1.89 and -1.94
Structure solved by	Freitag Mathias	Freitag Mathias	Freitag Mathias

Compound	**61 . CH_2Cl_2**	**62**	**65**
Identification code	Ict22	Ict20	Ict24
Empirical formula	$C_{21}H_{21}AuBrCl_2N_5O$	$C_{30}H_{26}AuClN_4O$	$C_{25}H_{31}AuBrN_5O$
Formula weight	707.20	690.96	694.42
Crystal system	Triclinic	Monoclinic	Orthorhombic
Space group	*P*(-1)	*C*2/c	$P2_12_12_1$
Crystal size [mm]	0.20 x 0.10 x 0.09	0.20 x 0.12 x 0.08	0.20 x 0.11 x 0.04
a (Å)	9.7277(5)	20.6859(3)	6.84802(15)
b (Å)	9.9275(5)	15.29914(12)	9.5220(2)
c (Å)	13.7791(8)	18.8633(2)	39.9463(8)
Volume ($Å^3$)	1203.06(11)	5246.80(10)	2604.76(9)
α (°)	70.949(5)	90	90
β (°)	83.029(4)	118.4908(17)	90
δ (°)	73.116(4)	90	90
Z	2	8	4
Density (calc.) [g/cm-3]	1.952	1.749	1.771
μ [mm^{-1}]	8.0	5.741	7.21
Temperature [K]	100(2)	100(2)	100(2)
Wavelength [Å]	0.71073	0.71073	0.71073
Theta range [°]	2.19 to 30.88	2.24 to 30.94	2.20 to 30.17
Reflections collected	40101	136619	109977
Independent reflections	7005	8056	7499
R1 with $I_0>2\sigma(I_0)$	0.0240	0.0207	0.0235
*w*R2	0.0495	0.0415	0.0478
G_0F	1.047	1.06	1.07
Largest diff. peak and hole [$e/Å^3$]	0.750 and -1.509	0.72 and -0.509	1.384 and -1.764
Structure solved by	Freitag Mathias	Freitag Mathias	Freitag Mathias

Compound	**84**	**85**	**90**
Identification code	ict04	cmx110	cmx111
Empirical formula	$C_{24}H_{30}ClN_4ORh$	$C_{62}H_{64}Ag_2F_{12}N_{16}O_4P_2$	$C_{34}H_{44}Br_2N_8O_5Pd$
Formula weight	528.88	1602.97	910.99
Crystal system	Orthorhombic	Monoclinic	Triclinic
Space group	$P2_12_12_1$	$P2_1/c$	P-1
Crystal size [mm]	0.40 x 0.04 x 0.02	0.07 x 0.05 x 0.02	0.20 x 0.10 x 0.03
a (Å)	7.1389(2)	21.5675(8)	9.6255(4)
b (Å)	13.2415(3)	22.9596(10)	16.5083(8)
c (Å)	25.0382(6)	14.4042(6)	23.9408(10)
Volume (Å^3)	2366.84(10)	6729.2(5)	3736.0(3)
α (°)	90	90	91.567(4)
β (°)	90	109.365(4)	95.478(4)
δ (°)	90	90	99.067(4)
Z	4	4	4
Density (calc.) [g/cm-3]	1.484	1.582	1.620
μ [mm^{-1}]	7.050	5.929	6.937
Temperature [K]	100(2)	100(2)	100(2)
Wavelength [Å]	1.54184	1.54184	1.54184
Theta range [°]	3.53 to 76.07	3.78 to 70.07	3.22 to 74.50
Reflections collected	55717	82550	49515
Independent reflections	4914	13960	15253
R1 with $I_0>2\sigma(I_0)$	0.0191	0.0516	0.0260
*w*R2	0.0487	0.1172	0.0679
G_0F	1.033	1.01	1.040
Largest diff. peak and hole [e/Å^3]	0.401 and -0.553	0.80 and -0.73	0.819 and -0.808
Structure solved by	Freitag Mathias	Daniliuc Constantin	Daniliuc Constantin

Compound	**91**	**96**
Identification code	cmx116	cmx113a
Empirical formula	$C_{68}H_{78}F_{12}N_{18}NiO_6P_2$	$C_{26}H_{30}Cl_2N_6O_2S_2Zn$
Formula weight	1592.13	658.95
Crystal system	Monoclinic	Orthorhombic
Space group	$P\ 2_1$	*Pnma*
Crystal size [mm]	0.26 x 0.26 x 0.19	0.18 x 0.14 x 0.03
a (Å)	18.4043(8)	10.983(2)
b (Å)	11.6515(4)	23.896(5)
c (Å)	19.0696(8)	11.349(2)
Volume (Å^3)	3817.0(3)	2978.4(10)
α (°)	90	90
β (°)	111.023(6)	90
δ (°)	90	90
Z	2	4
Density (calc.) [g/cm-3]	1.385	1.470
μ [mm^{-1}]	0.387	1.179
Temperature [K]	100(2)	100(2)
Wavelength [Å]	0.71073	0.71073
Theta range [°]	2.19 to 27.10	2.58 to 27.88
Reflections collected	131574	98658
Independent reflections	16815	3641
R1 with $I_0>2\sigma(I_0)$	0.0493	0.0349
*w*R2	0.1256	0.0682
G_0F	1.075	1.042
Largest diff. peak and hole [e/Å^3]	1.170 and -0.511	0.449 and -0.333
Structure solved by	Daniliuc Constantin	Daniliuc Constantin

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